

Project 1: *The Role of Mating-type Genes in Pathogenicity of Fusarium graminearum to Wheat*

1. What are the major goals and objectives of the research project?

- a. A comparative Illumina RNA-seq analysis of the wild type (WT) and KO transcriptomes in wheat heads, to reveal genes that are altered by activity of the heterodimeric mating specificity proteins versus by the non-dimerized forms.
- b. Cytological analysis of KO transformants expressing fluorescent proteins in inoculated wheat heads, to characterize the reduced aggressiveness of the MAT1-1-1 and MAT1-2-1 specificity gene KOs in detail.
- c. Produce complementation strains for each of the specificity gene KOs and confirm function in aggressiveness to wheat heads.

2. What was accomplished under these goals or objectives? (*For each major goal/objective, address items a-b below.*)

Objective 1.

- a) What were the major activities?

Protocols were developed and tested for production of high-quality RNA from infected wheat heads. Several preliminary experiments were performed to test yield and quality, and to optimize inoculation conditions and timing. Tissue was collected from the first replication of the experiment and flash frozen. COVID shut-downs prevented further work. A second wheat experiment will be planted in a few weeks and both experiments will be extracted and submitted for sequencing by the New Year.

- b) What were the significant results?

Optimized methods for inoculation and extraction of high-quality RNA were developed.

- c) List key outcomes or other achievements.

See above.

Objective 2.

- a) What were the major activities?

Wheat heads were inoculated with fluorescent wild-type *F. graminearum*, and numerous images were made with the confocal and the fluorescence microscopes. Work began on development of a new vector with the selectable marker nourseothricin for transformation of the mutant strains that are already hygromycin resistant.

b) What were the significant results?

Unfortunately, the quality of the imaging results has been fairly disappointing so far, due to very high levels of autofluorescence. Furthermore, COVID put a virtual stop to imaging from March-June due to the shut-down and associated maintenance issues. We will be getting a new confocal microscope soon that is specially equipped to eliminate autofluorescence and we will be attempting these experiments again after it arrives.

c) List key outcomes or other achievements.

In comparing our results with those of other reports, and with the general understanding of how *F. graminearum* invades spikelets we saw no evidence for invasion of the ovary, but only progression via the glume tissues into the rachis. One interesting finding was that the *F. graminearum* completely destroyed the phloem of the infected wheat tissues. After searching in the literature, we saw that others have reported a similar result, although it does not appear to have been much remarked upon.

Objective 3.

a) What were the major activities?

Work was begun to produce clones containing the selectable marker nourseothricin and the wild type mating type genes for complementation of the mutants.

b) What were the significant results?

The wild-type sequences were amplified using LR-PCR and sequenced. Further analysis of additional MAT mutants has revealed a large amount of variation among them, complicating our decision of which one to complement.

c) List key outcomes or other achievements.

Further analysis of additional MAT mutants has revealed a large amount of variation among individual transformants. We have continued work to characterize multiple KO strains of each type in an attempt to separate genuine activity of the KO from random variation.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

The University of Kentucky, including all research labs, shut down in March due to COVID, and all researchers were sent to work from home except for critical needs. We were allowed to come back ½ time beginning in June, and just this week we have been permitted to apply

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for “phase 3” reopening which will be 70% time. Naturally, this closure did impact our progress unavoidably. Since we were allowed to come back ½ time only in June, conditions in the summer greenhouse were not ideal for wheat and those experiments had to be delayed. We have been working in the laboratory but by necessity limited hours which has slowed our progress. We hope to be back up to speed very soon: phase 4 (return to 100% effort) is expected by the end of the semester.

4. What opportunities for training and professional development has the project provided?

Three students have worked on this project since its inception. In the first year one PhD student and one visiting scholar worked together on the project. Both have since graduated. An M.S. student began on the project in Fall 2019. All have had opportunities to learn protocols for *F. graminearum* inoculation and molecular manipulation, all have attended meetings and presented data.

5. How have the results been disseminated to communities of interest?

Results were presented at the USWBSI meeting in WI, the APS meeting in August 2019, and at the NC-1183 Multistate Committee meetings in November in VA, and virtually this past September. The student was invited to present at the International Fusarium Conference that was to occur in Banff in April but was cancelled due to COVID.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (9/1/19 - 8/31/20). The term “support” below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student’s stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY19 award period?**

No.

If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**

Yes

If yes, how many? Two. The first student worked on the project as a visiting scholar. The work made up the second chapter of her PhD dissertation, which she defended successfully last May. The second student was not directly supported (i.e. stipend), but worked together with the visiting scholar on the project, including conducting disease and mycotoxin ratings. That student earned his PhD in July.

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

NA

If yes, how many?

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

NA

If yes, how many?

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Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY19 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	Grain Class	FHB Resistance (S, MS, MR, R, where R represents your most resistant check)	FHB Rating (0-9)	Year Released

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the ‘Publications’ section of the FPR.

Abbreviations for Grain Classes

- Barley - BAR
- Durum - DUR
- Hard Red Winter - HRW
- Hard White Winter - HWW
- Hard Red Spring - HRS
- Soft Red Winter - SRW
- Soft White Winter - SWW

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (9/1/19 - 8/31/20)** should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/presentation.

Journal publications.

de Barros, A. V., Bec, S., Machado, F., Trail, F., Van Sanford, D.A., Alves, E., and Vaillancourt, L.J. 2019. The role of mating-type genes in pathogenicity of *Fusarium graminearum* to wheat. *Plant Health* 2019.

Status: Abstract Published and Poster Presented.

Acknowledgement of Federal Support: YES (Abstract and Poster)

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.

Bec, S., F.J. Machado, M. Farman, A. Vieira de Barros, S. Schwartz, R. Metz, C. Johnson, D.A. Van Sanford, E. Del Ponte and L.J. Vaillancourt. 2019. "Highly Aggressive and Toxigenic Transgressive Progeny from a Cross of Model *Fusarium graminearum* Strains PH-1 and GZ3639 are Associated with a Recombination Hotspot on Chromosome 2." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 61), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract published or Oral Presentation given

Acknowledgement of Federal Support: YES (Abstract and Poster)

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Poster Presentations:

Soto, G. E. Y., de Barros, A. V., Bec, S., Machado, F. J., Trail, F., Van Sanford, D., and Vaillancourt, L. (2019, December). "EXPLORING THE ROLE OF MATING-TYPE GENES IN *FUSARIUM GRAMINEARUM*." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 79), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract Published and Poster Presented.

Acknowledgement of Federal Support: YES (Abstract and Poster)