Project FY22-PB-003: Role of Chemotype in Aggressiveness and Toxigenicity of *Fusarium graminearum* to Wheat

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1. **What are the major goals and objectives of the research project?**
   The goal of our project is to test the hypothesis that the *F. graminearum* 3ADON chemotype confers higher levels of aggressiveness, toxigenicity, and competitiveness than the 15ADON chemotype, regardless of genetic background. The objectives are: **1)** Determine whether trichothecene toxin chemotype, mating type locus, and other genetic makers exhibit Mendelian segregation among progeny from crosses of strains from different *F. graminearum* populations; **2)** Determine whether individuals and mixtures of progeny from outcrosses differ in aggressiveness, toxigenicity, and competitiveness in susceptible and moderately resistant wheat in the presence and absence of fungicides; and **3)** Identify DNA markers associated with aggressiveness and high toxin production by analyzing whole genome sequence data from pools of progeny that differ in these traits.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*
   - **a.** For **Objective 1,** our goal is to test the hypothesis that crosses with Δ*mat1-1-1* tester strains will produce expected Mendelian segregation patterns of markers across all four chromosomes. The mapping populations we will generate as part of this objective will be stored as a permanent collection and made available to the community on request.
     - **i. What were the major activities?**
       1. We received a collection of 37 North American *F. graminearum* strains from wheat, representing four different chemotypes (15ADON, 3ADON, NIV, NX2)**.** These strains have been stored as a permanent collection on silica gel and in glycerol in our freezers.
       2. The chemotype of each of the strains was confirmed by PCR, and each strain was phenotyped for growth and sporulation *in vitro*, sexual fertility, and aggressiveness and toxigenicity on the susceptible spring wheat variety Wheaton to aid selection of the most suitable parents for our crosses.
     - **ii. What were the significant results?**
       1. The strains varied markedly in the traits analyzed. **We selected the crossing parents for this objective based on their growth *in vitro* and their aggressiveness to Wheaton**. Strains that grew abnormally or sectored frequently, produced few conidia, exhibited low levels of fertility in crosses with the tester, or had very low levels of aggressiveness, were removed from consideration as parents in the crosses.
     - **iii. List key outcomes or other achievements.**
       1. Three strains (3ADON strain **NRRL 46434**; NIV strain **NC016**; and NX2 strain **NRRL 66040**) were chosen as the most suitable parents for the crosses.
       2. An additional 3ADON strain, KY410, turned out to have very low aggressiveness and produced no detectable toxin in wheat heads. This result seemed interesting for future study, and so this strain was also selected for crossing.
       3. All four strains have been crossed with the 15ADON mating type gene deletion tester strain that was derived from PH-1. Progeny (264

from each) were collected, single-spored, and stored from each cross. A subset consisting of 40 progeny chosen at random was genotyped by PCR and expected Mendelian segregation patterns of the TOX and MAT loci were confirmed**.**

4. **This objective is completed.** The progeny collections can be made available to the community on request with appropriate permits.

b. For **Objective 2** our goal was to determine whether individuals and mixtures of progeny from the outcross of the 15ADON MAT- deletion tester strain and the more aggressive 3ADON parent (NRRL 46434**)** differ in aggressiveness, toxigenicity, and competitiveness in susceptible and moderately resistant wheat in the presence and absence of fungicides.

i. What were the major activities?

1. During the past year, most of our project effort has been devoted to this objective. Eighty progeny strains from our 3ADON/MAT+ vs 15ADON/MAT- cross (20 3ADON/MAT+: 20 3ADON/MAT-: 20 15ADON/MAT+: 20 15ADON/MAT-) were selected at random for pathogenicity assays on susceptible Wheaton and moderately resistant Alsen wheat varieties.
2. Three experiments, including 5 replications for each progeny strain plus parental strains in a RCB design, were completed for Wheaton. Unfortunately, the Alsen wheat did not thrive in our greenhouse, so only two experiments could be completed for that variety.
3. For the Wheaton and Alsen experiments, data analysis is complete for disease severity. Mycotoxin data are available for only one Wheaton and one Alsen experiment so far; analysis of the others is still in process.

ii. What were the significant results?

1. There was significantly less disease overall on the Alsen variety versus the Wheaton variety, as expected. For both wheat varieties, there was **no significant difference** in the aggressiveness of the 3ADON versus 15ADON progeny. There was also no significant effect of the MAT deletion.
2. The initial results of the mycotoxin analysis suggest that the mycotoxin profiles matched the PCR chemotype of the progeny (with only one exception, see #3 below), and the mycotoxin levels were positively correlated with the disease severity. There was also no major difference in the toxigenicity of the 3ADON versus 15ADON progeny in either wheat variety, but we still need to analyze the other experiments before we can make any final conclusions.
3. The single exception mentioned above appears to have been a rare heterokaryotic strain that included mostly 15ADON nuclei with a very small minority of 3ADON nuclei. It had originally been chemotyped as a 15ADON strain based on the PCR banding pattern. Interestingly, this strain produced primarily 3ADON in infected wheat heads, indicating that the 3ADON nuclei **MIGHT** be more competitive than the 15ADON. We will be follow up on this finding.
4. Nine progeny that were consistently more aggressive and toxigenic than the average (aka. “high” progeny), and 11 that were consistently less aggressive and toxigenic (aka. “low” progeny), were identified and confirmed in two additional greenhouse experiments on Wheaton with 15 replications per treatment. There was no

relationship between the chemotype and extreme high versus low aggressiveness or toxigenicity.

iii. **List key outcomes or other achievements.**

1. Although our analyses of the experiments for this objective are still incomplete, they already allow us to reject part of our original hypothesis, that 3ADON is specifically responsible for the high level of aggressiveness of the NRRL 46434 3ADON strain. Our data suggest instead that there are multiple factors segregating in the cross that contribute to aggressiveness.

c. For **Objective 3**, our goal was to conduct genome-wide SNP (single nucleotide polymorphism) analysis of bulked progeny pools from the cross of the 3ADON and 15ADON chemotype. The aim is to identify new genetic markers associated with aggressiveness, competitiveness, and toxigenicity.

i. **What were the major activities?**

1. Genome sequence assemblies for all the selected parental strains were generated in the Proctor laboratory at the USDA-ARS in Peoria. For genome sequencing, strains were first grown on a growth medium amended with antibiotics to remove any potential bacterial contamination. Genomic DNA was isolated from liquid cultures using the Qiagen DNeasy Plant Mini Kit. Resulting DNA was then subjected to a 16S PCR screen to confirm the absence of bacterial DNA, and then used to prepare libraries for sequencing with an Illumina MiSeq instrument. Resulting sequence reads were processed with the computer program CLC Genomics Workbench. Adapter and low-quality sequences were removed from sequence reads, and then reads were screened against 73 bacterial genome sequences to remove low levels of bacterial sequence reads resulting from contaminated reagents and/or equipment. The unmapped sequences were then used to generate an assembled genome sequence using CLC Genomics Workbench.
2. The genomes of the the parental strains were aligned with each other and with a set of *F. graminearum* genome assemblies from strains representing the three major North American populations (NA1, NA2, NA3) (https://doi.org/10.1371/journal.pone.0194616). SNPs were identified with "iSNPcaller" (https://github.com/drdna/iSNPcaller).
3. The high-low strains from the 3ADON vs 15ADON cross (see above) have been sent to the Proctor laboratory for sequencing. Although we had originally planned to sequence bulked pools of the high and low progeny, a new method became available in the Proctor lab that will allow us to sequence the genomes of the strains individually. This work is now in progress.

ii. **What were the significant results?**

1. Genome assemblies for the parent strains ranged from 33 to 36.7 Mb in size and included 393-593 contigs (**Table 1**). The number of SNPs differentiating the parent strains from the 15ADON *Mat1-1-1* deletion strain ranged from 120,517 to 166,083. There were 144,116 SNPs that distinguished the 3ADON NRRL 46434 and 15ADON parents (**Table 1**).

iii. **List key outcomes or other achievements.**

1. Comparisons of the genome assemblies to representatives of the three North American subpopulations (NA1, NA2, and NA3)

indicated that NRRL 46434 belongs to the NA2 population and NRRL 66040 belongs to the NA3 population as expected based on their chemotypes. The NC016 strain hasn't been characterized yet.

2. Interestingly, the KY410 strain, which chemotyped as 3ADON in the PCR assay but was not highly aggressive or toxigenic in the wheat assays, is an apparent hybrid between the NA1 and NA2 populations. Furthermore, its TOX genes are identical to those of the NX2 chemotype that was previously known only from the NA3 population. This strain also produced NX2 in *in vitro* assays (Note that our PCR assay cannot distinguish NX2 from 3ADON: however, it is still suitable for marker analysis of progeny from crosses of known parental strains. Further, the 3ADON chemotype of the NRRL46434 strain, unlike the KY410 strain, was confirmed independently by mycotoxin profiling of diseased wheat heads).

TABLE 1: Assembly Statistics for the *Fusarium graminearum* Genomes Sequenced For This Project

	PH-1[1]	NRRL 66040	NCO16	NRRL 46434	KY410
Number of Contigs	433	460	593	393	534
Assembly Size (Mb)	36.2	36.7	33.0	36.5	36.6
N50 (kb)	184.6	201.6	138.3	203.5	179.3
Number of SNPs[2]	2662	166083	137729	144116	120517

1. PH-1 information was copied from GenBank.
2. Number of SNPs differentiating alignment from the Mat1-1-1 deletion tester strain, which was derived from PH-1

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

The M.S. student on this project joined us in January 2023, with an excellent background in agronomy and plant breeding from her undergraduate studies. During the past 18 months she has made outstanding progress in her degree, completing all her course requirements and mastering all the relevant molecular and pathological assays for our project, thus developing valuable new knowledge and skills in fungal genetics and phytopathology. She recently completed the Fusarium Laboratory Workshop in Manhattan Kansas which deepened her practical knowledge of the *Fusarium* genus and provided an opportunity for her to make new professional connections. She has learned R and Linux programming so she can work with her genome data (Objective 3). When she completes her M.S., she will have a valuable combination of knowledge and skills relevant to crop improvement from both the host and the pathogen side.

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

The student presented a research poster at the USWBSI forum in Cincinnati in December 2023, and she also gave a talk at the NC1183 mycotoxins committee annual meeting that was held at NCAUR in Peoria, Illinois in May 2024.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

a. **Work for objective 1 is completed.** We plan to create an online database listing the strains and the available information on their phenotypes that can be disseminated to the community.

b. **We have made good progress on work for objective 2.** Next year we will be conducting additional greenhouse experiments with mixtures of high-low and other progeny in the presence and absence of fungicides to better understand the

relationship between aggressiveness and competitiveness, and to identify additional isolates that exhibit strong competitiveness for our marker association analysis in Objective 3.

c. **We will be focusing more on objective 3 in the next year**: Work with the high-low progeny will focus on identifying markers linked to aggressiveness, toxigenicity, and competitiveness. For this we will be identifying SNP markers that vary from the expected 1:1 ratio among segregating progeny. We will also be investigating evidence for recombination hot and cold spots, and working to better understand the overall process of recombination in outcrosses of *F. graminearum*.

d. We will present our results as a poster at the Annual Forum of the USWBSI in Austin, Texas, next December. We will be drafting our first publication, focused on the role of the 3ADON chemotype in aggressiveness, competitiveness, and pathogenicity, by the end of the year.