Project 1: 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.

   What were the major activities?
   Research for Objective 1 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details.

   What were the significant results?
   Research for Objective 1 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details.

   List key outcomes or other achievements.
   Research for Objective 1 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details.

   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.

   What were the major activities?
   Research for Objective 2 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details. However, Dr Proctor provided input on the analyses of the progeny, and arranged to have toxin chemotypes of progeny analyzed in the laboratory of Susan McCormick at USDA ARS NCUAR.

What were the significant results?
Research for Objective 2 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details.

List key outcomes or other achievements.
Research for Objective 2 was done in the Vaillancourt laboratory at the University of Kentucky. Please see Dr. Vaillancourt's report for details.

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.

What were the major activities?
1. Genome sequence assemblies for the parental strains selected for this project were generated using an Illumina MiSeq Instrument. To generate the sequences, strains were 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 MiSeq libraries. 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 remaining sequences were then used to generate an genome sequence assembly using CLC Genomics Workbench.

2. The Vaillancourt Lab sent 20 progeny from their cross of a 3ADON-producing strain (NRRL 46434) to a 15ADON-producing strain (a *mat1-1-1* deletion mutant derived from strain PH-1). Genomic DNA from each progeny has been generated using the protocol described in the previous section. Genome sequences are schedules to be generated from the DNA in August 2024 using an Illumina NextSeq instrument. Although we originally planned to sequence pools of progeny, the NextSeq instrument has made it cost-effective to generate genome sequence data for each progeny individually.

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).

Table 1: Assembly statistics for genome sequences from strains of *Fusarium graminearum* generated and/or used in this project[1].

	PH-1	NRRL 66040	NRRL 46434	NCO16	KY410
Number of Contigs	433	460	393	593	534
Assembly Size (Mb)	36.2	36.7	36.5	33.0	36.6
N50 (kb)	184.6	201.6	203.5	138.3	179.3

[1] Statistics for the PH-1 genome sequence were obtained from NCBI/GenBank. All other information was derived from an in-house genome sequence database at USDA ARS NCAUR. .

List key outcomes or other achievements.

1. The Vaillancourt lab used a whole-genome SNP analysis to assess the relationships of the parental strains selected for this project (KY410, NCO16, NRRL 46434, NRRL 66040, and PH-1) to the North American populations of *F. graminearum*. To complement the SNP analysis, the Proctor lab retrieved sequences of 12 housekeeping genes and used a phylogenetic analysis (i.e., maximum likelihood tree inference) to assess the relationships of the of the parental strains to reference strains from the five North American populations of *F. graminearum*: NA1, NA2, NA3, Gulf Coast and Southern Louisiana. Consistent with the results of the SNP analysis, the maximum parsimony analysis indicated that the 3ADON parental strain (NRRL 46343) is most closely related to reference strains from the NA2 population, and that the NX parental strain (NRRL 66040) is most closely related to reference strains from the NA3 population. The parental strains KY410 and NC016 group within a clade consisting of NA1 and NA2 reference strains, but the topology of the tree and bootstrap values of the branches do not provide evidence that KY410 and NC016 are more closely related to NA1 or NA2, which indicates that they could be hybrids between two populations.

   Figure 1: Maximum likelihood tree inferred from the DNA sequences of 12 housekeeping genes retrieved from the parental strains used in this project and selected reference strains from each of five North American populations of *F. graminearum*: the NA1, NA2, NA3, Gulf Coast, or Southern Louisiana populations. The genes have been used extensively in phylogenetic analyses of *Fusarium* and are indicated in Table 2. The parental strains used in this project are highlighted in blue. All other strains included in the tree are reference strains that have been previously determined to members of one of the five populations. All strain designations consisting of 5 digits are NRRL strains. For simplicity, the NRRL prefix has been removed.

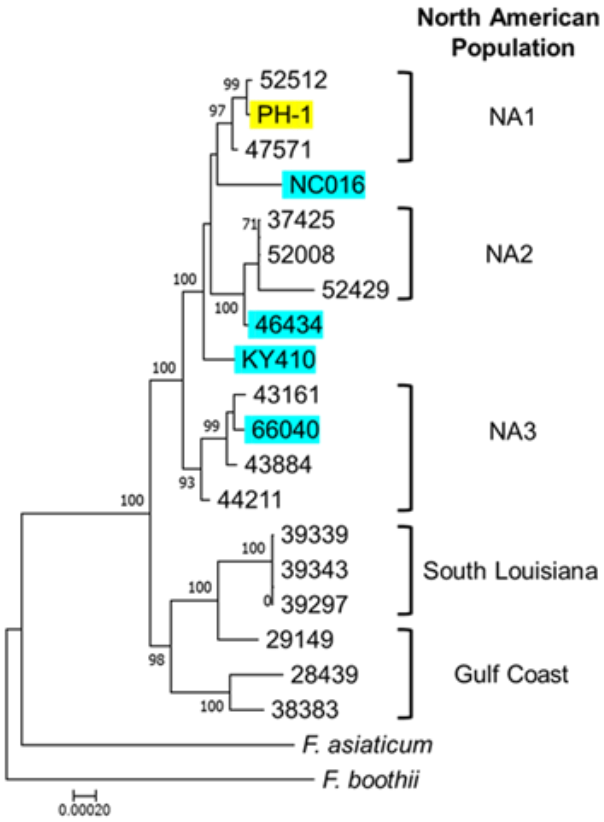

Table 2: Housekeeping genes used in phylogenetic analysis of parental strains of *F. graminearum*.

No.	Gene	Corresponding Protein	Alignment Length
1	*ACL1*	ATP citrate lyase large subunit	1835
2	*ACT1*	Actin	1490
3	*CPR1*	Cytochrome P450 Reductase	2201
4	*DPA1*	DNA Polymerase Alpha Subunit	4877
5	*DPE1*	DNA Polymerase Epsilon Subunit	6885
6	*FAS1*	Fatty Acid Synthase Alpha Subunit	5682
7	*MCM7*	DNA Replication Licensing Factor	2649
8	*PHO5*	Phosphate Permease	1852
9	*RPB1*	RNA Polymerase Largest Subunit	5383
10	*RPB2*	RNA Polymerase 2nd Largest Subunit	3911
11	*TEF1*	Translation Elongation Factor 1-alpha	1768
12	*TSR1*	Ribosomal biogenesis protein	2507

2. Various combinations of functionally different alleles of the trichothecene biosynthetic genes *TRI1*, *TRI8* and *TRI13* determine whether *F. graminearum* strains produce 3ADON, 15ADON, NIV or NX. To confirm that the parental strains selected for this project have a combination of *TRI* gene alleles that correspond to their PCR-based chemotypes, we retrieved the DNA sequences for the three genes from the genome sequences of each parental strain and then compared the

gene sequences to those of reference strains. This analysis confirmed that the parental strains selected for this project had *TRI* gene alleles consistent with their previously determined chemotypes. Although sequences of *TRI1*, *TRI8* and *TRI13* were analyzed, only results from analysis of *TRI8* are shown (Figure 2).

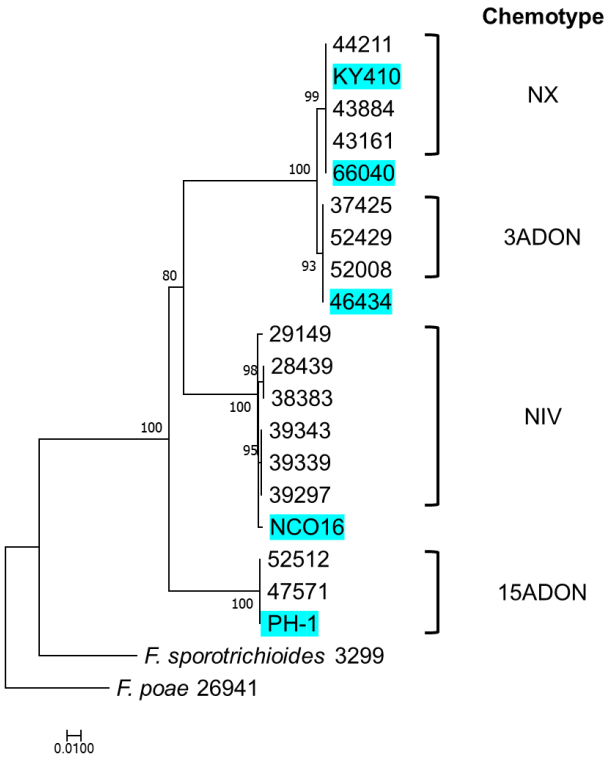

Figure 2: Maximum likelihood tree inferred from the DNA sequence of the trichothecene biosynthetic gene *TRI8*. The parental strains used in the study are highlighted in blue. All other strains included in the tree are reference strains for which alleles and chemotypes have been confirmed previously. Although different *TRI8* alleles confer the structural differences between 3ADON and 15ADON, sequence variation in the gene makes it a good marker to differentiate among the four common chemotypes reported in *F. graminearum*: that is, production of 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), nivalenol (NIV) or NX. All strain designations consisting of 5 digits are NRRL strains. For simplicity, the NRRL prefix has been removed.

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

Dr. Vaillancourt has assigned an M.S. student to this project, and the student began in January 2023. For details about student, please Dr. Vaillancourt's performance report. Dr. Proctor has frequent interactions with the student via emails and, less frequently, at meetings.

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

The M.S. student presented a poster on this research at the USWBSI National FHB Forum in Cincinnati in December 2023. She also gave an oral presentation at the NC1183 mycotoxins committee annual meeting at USDA ARS 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?

To address **Objective 3**, the Proctor lab will continue to generate genome sequence data for *F. graminearum* progeny produced in the Vaillancourt lab. The resulting data will be transferred to the Vaillancourt lab and analyzed by the M.S. student. The Proctor lab will also analyze *TRI* gene sequences in the progeny to confirm chemotypes of the progeny.