Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports
Format

(Begin 1 page limit)
Project #:3019-3564

Progress Report Year: _3___ *of* _3___ *(maximum of 3 year funding cycle)*

Title: **Fusarium Crown Rot on Wheat: Prebreeding and Development of Tools for Genetic Disease Management**

M. Pumphrey, K. Garland-Campbell, and T. Paulitz

Cooperators:

Executive summary:
- we found two potential QTLs for resistance to *F. culmorum,* in addition to the two found to *F. pseudograminearum* in a previous funding cycle.
-we have screened almost 500 lines from regional nurseries and variety testing for resistance to *F. culmorum,* and are identifying the most resistant and susceptible.
-we have further optimized our greenhouse testing protocol to maximize disease and reduce variability by using a cold vernalization period followed by a water stress treatment at the end.
-we assessed lines in five variety testing sites in the intermediate rainfall zone for two years, in areas with high levels of *F. culmorum*. However, results were confounded by eyespot
-We conducted inoculated field trials for three years in Lind and Pullman, and have identified the most susceptible and resistant
-we evaluated statistical designs and models to minimize spatial variation in the WA variety testing locations, and found a better model than the one presently used.

Impact:
The economic impact of this disease continues to be large and impacts all growing areas of Washington.
What measureable impact(s) has your project had in the most recent funding cycle?
A list of the most susceptible and resistant varieties, better methods to screen in the greenhouse for future work.

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Fusarium Grant 2015-2017

3019-3564

Fusarium Crown Rot on Wheat: Prebreeding and Development of Tools for Genetic Disease Management

M. Pumphrey, K. Garland-Campbell, and T. Paulitz

3 year summary and final report.

Over the last 3 years, our main objectives have been to identify new and existing sources of resistance that can be use in the WA breeding programs to create tolerant varieties. Finding resistance to Fusarium crown rot is a much more intractable problem than finding single-major genes to control diseases such as stripe rust or cereal cyst nematode. There are no major genes identified for resistance against this disease. A much longer and better funded effort in Australia over the last 30 years has identified some varieties with moderate tolerance. They also have identified the most susceptible varieties, which is another goal of our program. The Australians have been able to obtain better resistance by combining sources of partial resistance. The other difficulty is the large genotype X environment interaction with this disease, which makes field screening much more difficult. Thus, much of our effort has gone into perfecting a reliable greenhouse screening method, and we have recently made advances in getting higher disease levels by vernalizing and water stressing the plants and rating them at the boot stage rather than at the seedling stage. Unlike the previous 3 year cycle, we have also concentrated on *F. culmorum* rather than *F. pseudograminearium.* This species is also widespread in the PNW and gaps needed to be filled in.

Our main objectives have been

1) to find new sources of resistance with association mapping. This is a continuation of previously funded projects to find QTLs to *F. pseudograminearum.* The efforts of our PhD student Yvonne Thompson is to conduct a genome-wide association study (GWAS) to determine the genetic architecture of resistance to *F. culmorum* in a diverse global spring wheat collection. To achieve this objective, a modified greenhouse screening system* was developed that encourages higher disease pressure enhancing our capability in evaluating disease response. The germplasm used is part of the spring core nursery of the USDA National Small Grains Germplasm bank. A representative subset of 600 wheat accessions, from nearly 5,000, was obtained via phylogenetic analysis. This collection was screened three times in 2015-2016. Resulting average ratings of the three trials were highly skewed towards resistance suggesting that disease pressure was too low to separate resistant from susceptible wheat accessions. As a result, the screening system was reassessed and modified to produce the optimal environment for pathogen growth and infection. Using the modified greenhouse screening system, phenotypic data of one 2017 trial suggest differential responses reflecting genetic variability under disease pressure. Genotypic data for the germplasm set was acquired using the Illumina wheat 9K iSelect SNP chip which has also been assayed for several other field and disease related traits as part of the USDA-NIFA Triticeae CAP project. Thus, it can be an important resource where a number of resistant genes can be combined. GWAS was conducted using the software package GAPIT

R. Two statistically significant marker trait associations (MTA) were confirmed on chromosomes 3B and 2B, however more phenotypic repetitions are needed to increase experimental power. Results from repetitions 2-4 are currently underway and will be available for analysis in February 2018. KASP markers will be developed and verified from MTAs for use in assisted selection for introgression into wheat cultivars ultimately providing improved resistance to Fusarium crown rot.

*Modified Greenhouse Screening System

Five PNW *F. culmorum* isolates were used in the assays to include a wide range of pathogenic capabilities representative of isolate variability in the native population. A colonized grain method was used to produce inoculum. Inoculated trials were conducted in a Conviron growth room provided by the Washington State University Plant Growth Facilities. Growth room temperature was set at 10°C during the day and 5°C at night with a photoperiod of 14-hour days and 10-hour nights during the first week. Thereafter, the temperature was adjusted on a weekly basis to correlate with optimum fungal toxin production as follows.

Temperature adjustment by week for growth room		
Week	Day Temp in C	Night Temp in C
1	10	5
2	13	8
3	15	10
4	18	13
5	20	15
6	25	20
7	30	25
8	35	30

Method: A single seed from each accession was planted in a 4 cm diameter 20.5 cm long cone-tainers (Stuewe and Sons, Corvallis, OR, USA) arranged in racks and filled with Sungro professional growing mix (Sun Gro Horticulture Inc, Bellevue, WA, USA). One-week-old seedlings were inoculated with one gram of colonized millet. Plants were watered over the top for the first 2 weeks then sub-irrigated every other day with regular water during the week and nutrient water during the weekend. Plants were subjected to water stress two weeks prior to rating, to exacerbate disease symptoms, by watering at wilt only. Stem base crown tissues of eight-week-old plants were rated and recorded for disease severity on a 0-10 rating scale (0 meaning no infection and 10 meaning severely diseased).

The second object was **to screen variety collections and regional nurseries in the greenhouse.**

This includes both spring and winter material. Unlike the previous objective which looks for exotic sources of resistance, the purpose of this objective is to identify the level of resistance or tolerance in existing lines. Over the last 3 years over 407 lines have been rated in 8 greenhouse trials. These involve inoculation with a mixture of *F. culmorum* isolates and rating the crown rot on a 1-10 scale. However, in 2016, we optimized this test by giving the spring wheat a cold treatment the first two weeks at (4 C) to simulate conditions in the field and then to water stress the plants at the end. Water stress has been known for many years to be a predisposing factor to this disease, but is often difficult to do in the greenhouse. We attempted to mimic water stress by providing fertilizer and adequate water for plants to grow at their full potential. In the last two weeks of the trial, plants were watered only twice a week. This caused the plants to weaken and wilt, allowing the Fusarium to take advantage. Since 2016, we have further optimized the test to the aforementioned conditions (*Modified Greenhouse Screening System) which produces consistent and more severe Fusarium crown rot disease.

Dylan Larkin, the MSc student funded by this project, rated 106 soft white winter wheat lines using nine replicates grown under controlled conditions and inoculated with a mix of five *F. culmorum* isolates collected throughout Eastern Washington. Genotypes were evaluated for crown rot severity on a 1 through 8 scale, with 1 = no disease and 8 = most susceptible. The distribution of average disease symptoms ranged from 2.4 to 5.4. There were 22 genotypes significantly more resistant than the susceptible check 'Madsen', while nine of those were significantly more resistant than the partially-resistant check 'Bobtail'. The named varieties that were more resistant than Bobtail were Xerpha, SY Assure, Eltan, SY Dayton, and Northwest Tandem. Susceptible lines included Madsen, Legion, and AP700CL. 23 lines were significantly more tolerant to FCR compared to the susceptible check, 'Madsen' ($\alpha = 0.05$). Additional spring wheat testing is needed but more resistant lines include Otis, WA8163, and SY605CL.

3. The third objective was **to test varieties in the field, either in inoculated trials or under natural inoculum.**

Field experiments at the Reardan, Creston, Lamont, Ritzville, Mansfield, Harrington, and Connell variety testing locations in 2015 and 2016 containing the same 106 soft white wheat genotypes tested above were evaluated for symptoms of Fusarium crown rot. These areas were chosen because it is a hot area for *F. culmorum.* There were positive correlations among genotype ratings for response to Fusarium crown rot between Lamont and Ritzville, Reardan and Harrington, and Lamont and Harrington. Symptoms of crown rot were identified and rated in all environments. Field results were highly variable due to differing environmental conditions from 2015 to 2016 and presence of other soil-borne pathogens.

Correlations between field and greenhouse screening trials were weak, likely due to the presence of other pathogens, variation in soil type, FCR disease severity and climatic factors. The pathogen most likely to cause a discrepancy in field screening is eyespot or strawbreaker foot rot, caused by *Oculimacula* spp., which is widely distributed in the dryland wheat areas of the Pacific Northwest. The crown symptoms are similar, except that eyespot also causes characteristic 'eye-shaped' lesions on the stems. The two pathogens are widely distributed in all wheat production locations and the one causing the most disease is likely due to soil moisture availability with Fusarium favored in drier environments.

We also tested spring varieties under inoculated conditions in Lind in 2015 and 2016 and in Pullman in 2017. At Lind, we manipulated the disease pressure by irrigating the wheat to provide luxurious growth, and then cutting the water to provide water stress.

Louise and Otis have consistently shown the lowest scores for Fusarium crown rot in these inoculated trials. These results enable us to use adapted breeding lines as a base to incorporate new sources for better combined resistance.

The other breakthrough of the work of Dylan was to test new statistical models to control for spatial variation in the Washington State University Extension Cereal Variety Testing

Program, specifically linear mixed models with spatial covariance structures (SLMM). This was supported by the WGC and is an important spinoff of his Fusarium work. He evaluated yield data from 143 environments over 22 locations, seven years, and five precipitation zones using five different SLMM compared to the randomized complete block (RCB) and alpha-lattice designs (PBLR). Using Akaike Information Criterion and likelihood ration tests, he found that SLMM performed better in 86% of environments compared to RCB and PBLR designs. This information can be used for WSU and regional breeding programs in performing more efficient and effective analyses of their field data.

Deliverables

1. Resistance ratings of spring and winter varieties grown in WA
2. Identification of new QTLs for resistance to *F. culmorum*
3. Better greenhouse methodology for screening for Fusarium resistance in greenhouse
4. Better statistical design for variety testing trials

Refereed papers

Thompson, AL, Mahoney, AK, Smiley, RW, Paulitz, TC, Hulbert, S, Garland-Campbell, K, 2017. Resistance to multiple soil-borne pathogens of the Pacific Northwest is co-located in a wheat recombinant inbred line population. G3: Genes, Genomes, Genetics. 7:1109–1116

Theses

Larkin, D.L., 2017. Disease screening and statistical strategies for predicting variety performance in wheat. Masters Thesis. Dept. of Crop and Soil Sciences, WSU. Pullman WA.

Abstracts

Thompson, Y.A., Garland-Campbell, K.A., Paulitz, T.C., 2016. Differential Response of Wheat (*Triticum aestivum* L.) to *Fusarium culmorum*. Poster session presented at: Resilience emerging from scarcity and abundance. ASA, CSSA and SSSA International Annual Meetings, Phoenix, AZ. 6-9 Nov. Poster 332-906.

Thompson, Y.A., Garland-Campbell, K.A., Paulitz, T.C., 2017. Genome wide association study (GWAS) in spring wheat to identify QTLs for resistance to *Fusarium culmorum*. Poster session presented at: Diverse Crops-Diverse Challenges. National Association of Plant Breeders Annual Meeting, Davis, CA. 7-10 Aug. WED47.

Larkin, D.L, K.A. Garland Campbell, and T.C. Paulitz. 2016. Comparison of greenhouse and field rating systems for Fusarium crown rot in winter wheat. Poster session presented at: Resilience emerging from scarcity and abundance. ASA, CSSA and SSSA International Annual Meetings, Phoenix, AZ. 6-9 Nov. Poster 163-1417.

Larkin, D.L., K.A. Garland Campbell, and T.C. Paulitz. 2016. Comparison of greenhouse and field rating systems for Fusarium crown rot in winter wheat. Poster session presented at: Improving efficiency in breeding programs. NAPB Annual Meeting, Raleigh, NC. 15-18 Aug. Poster 29.

WGC project number:	**3019-3564**
WGC project title:	**Fusarium Crown Rot on Wheat: Prebreeding and Development of Tools for Genetic Disease Managemen**
Project PI(s):	**M. Pumphrey, K. Garland-Campbell, and T. Paulitz**
Project initiation date:	**7/1/2015**
Project year:	**Year 3 2016/2017**

Objective	Deliverable	Progress	Timeline	Communication
Objective 1. Perform association mapping on spring and winter core collections to identify further sources of resistance.	Resistant sources that can be used for variety development.	A mini-core collection was developed for the spring core collection. Phenotyping and screening of mapping population in the greenhouse has been completed. We found two potential QTLs for resistance to *F. culmorum*, in addition to the two found to *F. pseudograminearum* in a previous funding cycle.	Verification of QTLs will continue in 2018-2019, see new proposal. In addition, we will be testing a new CIMMYT synthetic population in 2018-2019	See publication list
Objective 2. Screen all variety and regional nurseries for resistance in greenhouse screening.	Ratings of varieties for Fusarium tolerance in the the WSCIA seed buyers guide and other publications.	We have screened almost 500 lines from regional nurseries and variety testing for resistance to *F. culmorum,* and are identifying the most resistant and susceptible. We have further optimized our greenhouse testing protocol to maximize disease and reduce variability by using a cold vernalization period followed by a water stress treatment at the end.	Greenhouse screening will continue with optimized methods in 2018-2019	See publication list
Objective 3. Expand field testing to two locations, and test variety and regional nurseries.	Ratings of varieties for Fusarium tolerance in the the WSCIA seed buyers guide and other publications.	We assessed lines in five variety testing sites in the intermediate rainfall zone for two years, in areas with high levels of *F. culmorum*. However, results were confounded by eyespot -We conducted inoculated field trials for three years in Lind and Pullman, and have identified the most susceptible and resistant -We evaluated statistical designs and models to minimize spatial variation in the WA variety testing locations, and found a better model than the one presently used.	All future field work will be with inoculated field plots in Lind and Pullman.	See publication list