

## Fusarium Grant 2018-2020

3019-3685

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

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#### 3 year summary and final report.

Over the last 3 years, our main objectives have been to continue identify new and existing sources of resistance that can be used 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 and field screening methods, 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. pseudograminearum*. This species is also widespread in the PNW and gaps needed to be filled in.

The main thrust of this research the last 3 years has focused on overcoming the two main obstacles with this disease.

1). **Consistent, accurate, and reproducible methods of phenotyping this disease.** Unlike foliar diseases that are easy to rate and quantify, Fusarium crown rot symptoms are more difficult to rate and highly dependent on environmental conditions. To identify the quantitative (minor gene) sources of resistance, we need phenotyping data that will be consistent and show enough range of resistance or susceptibility. We have focused on inoculated greenhouse methods, and found that by vernalizing the lines, and adjusting temperature and water stress, we could get more consistent results. We are still optimizing this method by trying to standardize inoculation, by using ground millet seed instead of whole seed, and quantifying the pathogen with dilution planting.

The other challenge has been developing field screening methods. Fusarium crown rot resistance can be expressed in seedlings in the greenhouse, but to be effective in the field, they will need to express adult plant resistance. These methods have evolved over the last 20 years. Initial studies by just rating disease in variety testing sites and relying on natural inoculum has not been effective, because of the strong genotype X environment interactions. This is a disease driven by drought and water stress, and some years resistance would not be present. It also assumed uniform inoculum in the field. In the previous funding cycle, we evaluated disease on varieties in variety testing sites at Harrington, Reardan, Creston, Ritzville, Harrington, Lamont

and St. Andrews. An MSc student developed statistical methods to optimize accounting for variance, but there were strong confounding effects with eyespot, which has symptoms that can be mistaken for Fusarium crown rot. Another approach, taken by Richard Smiley and being continued by Christina Hagerty, is to apply inoculum in paired rows in the field. This method is very time consuming and is limited to evaluation of only a few lines. In this funding cycle, we optimized a method at Lind of using durum wheat the previous year to build up natural inoculum. Durum is highly susceptible to Fusarium crown rot. The durum wheat is seeded early grown with trickle irrigation and fertilizer to produce large plants and then killed just after heading. The spring wheat cultivars are planted using no-till into the durum stubble the following spring. Alternate sides of the field are used each year. This is starting to produce good screening results.

**2) Identifying sources of resistance.** This disease does not have major gene resistance, unlike stripe rust, eyespot and cereal cysts nematode. We have worked with a number of populations that encompass a large degree of genetic variation and possible resistance. These are outlined in more detail under the objectives. These include

- a. A large Spring Wheat CAP population, with over 3000 lines. We selected a mini-core of 600 by analyzing genetic diversity, and conducted GWAS to identify QTLs effective against *F. culmorum*.
- b. a set of facultative synthetic wheat germplasm developed by CIMMYT in Turkey.
- c. Populations developed by backcrossing Iranian landrace AUS28451 to Louise.
- d. Doubled haploid population from Cara X Xerpha cross.
- e. The DNAM recombinant inbred lines developed by direct crosses between a hard white winter wheat and the wheat wild relative, *Aegilops tauschii*, that is the progenitor of the D genome of wheat.
- f. A synthetic population crossed to Louise about 10 years ago, and developed for resistance to Rhizoctonia

**Our main objectives have been to**

**Objective 1. Screen spring and winter variety trials and breeding lines for resistance in the greenhouse.** The quickest way to get resistant varieties into growers' hands is to have a robust screening system to handle all the advanced material developed by our breeders. Over the last 2.5 years, we have screened over 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 are reducing variation from run to run by more accurate quantification of the *F. culmorum* inoculum. **For the first time, we have a released variety with a high degree of tolerance to Fusarium crown rot, as a result of our Fusarium screening.** This variety, Devote, is a soft white winter wheat adapted to low to intermediate rainfall areas. It can emergence from deep planting, but

most importantly Devote combines strawbreaker foot rot resistance, snow mold resistance and tolerance to Fusarium. Strawbreaker foot rot and Fusarium crown rot often occur in a complex.

### **Objective 2. Select for QTLs associated with resistance in segregating populations**

As part of the previous cycle, we screened the spring wheat 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. These were genotyped with the Illumina wheat 9K iSelect SNP chip and phenotyped in a number of greenhouse trials, using our optimized screening method. These results have been submitted for publication. The phenotypic analysis revealed consistent resistant responses from breeding lines PI415182 and PI317693, landraces PI268468 and PI626634, and cultivar PI283872 with an average rating value of 2 or lower. Marker trait associations were identified on chromosomes 1AB, 2AB, 4D, and 5AB.

We developed a doubled haploid population from Cara/Xerpha which was evaluated in the greenhouse. This was the MSc work of Carmen Swannack, and was included as supplemental work, even though her main work focused on stripe rust and end use quality. Fifteen lines showed lower disease ratings than Xerpha and Madsen. These lines can be introgressed directly into adapted soft white winter breeding lines.

Another source of resistance is from a DNAM population, specifically from the *A. tauschii* parents U6713 and UC6716. Because these populations are only segregating for variation in the D-genome, we likely have identified novel resistance. This population was highly skewed toward resistance, with 32 progeny with Fusarium ratings below 2 on a 1-10 scale where 1 is the best.

### **Objective 3. Look for new sources of resistance in a new set of synthetic wheat that was developed by CIMMYT in Turkey.**

We received this set of facultative synthetics in 2017 from the International Wheat Improvement Program with CIMMYT in Ankara, Turkey and spent a year increasing the seed. These synthetics recreate hexaploid wheat and have been selected to have multiple disease resistance traits. Synthetic wheat is derived from crosses between durum wheat and *Aegilops tauschii*, the donor of the wheat D genome. Since durum is susceptible to Fusarium, any resistance that we identify is from the D genome. This germplasm represents potential new sources of resistance and has been crossed with winter wheat breeding lines from the USDA and WSU breeding programs. Over the last three years, we have crossed these to spring wheat breeding lines and we will cross them to winter wheat breeding lines. Populations derived from accessions with resistance to Fusarium crown rot will be prioritized to transfer their favorable characteristics. We can breed faster with spring wheat and then transfer the best-adapted combinations to winter wheat.

The adapted parents included the spring wheats Chet, Ryan, DH11SRW070-14, and the winter wheats Selbu, WA8252, Sequoia, and club wheat Cara. We chose good breeding lines from each market class and some of them have been rated as tolerant to Fusarium. These have been crossed with the two most resistant synthetics, SYNT-INT 15 and 16. These are currently at the BC1F2 stage. These have been screened in the greenhouse and are highly skewed toward resistance, with 37 progeny with rating below a score of 2.

We have also screened another set of synthetic line crosses, originally developed over 10 years by Scot Hulbert's program. These were crossed with Louise and screened for resistance to Rhizoctonia, presently the PhD work of Jade Emerita. These were screened for Fusarium crown rot resistance and were highly skewed to resistance to Fusarium crown rot. This population is more adapted, at backcross 5, and is much closer to Louise than the other synthetics, so should be easier to cross to adapted material.

#### **4. Screen the population of AUS28451 X Louise in the greenhouse for tolerance to Fusarium, to identify new possible sources of resistance.**

As part of an MSc thesis (A. Waziri) and previous PhD thesis (Alison Thompson), a mapping population was developed composed of 197 lines descended from a backcross between the Iranian landrace AUS28451 and Louise. This AUS28451 landrace was previously investigated as part of the PhD thesis of Alison Thompson, and showed resistance to multiple pathogens, including root lesion nematode, Fusarium and Rhizoctonia. The population was descended from six individual BC1F1 plants and was in the BC1F9 stage when we began evaluation. In 2017 and 2019, she rated the population and check cultivars for both adult and seedling resistance in the greenhouse and for adult resistance in field screening data. In the greenhouse, higher disease was seen in the adult plant assay than the seedling assay. The 2019 field trial was planted into land previously cropped with durum, which is highly susceptible to Fusarium. We had very high disease pressure in the field nursery in 2019.

The population was genotyped using 2300 single nucleotide polymorphisms (SNP) markers but many of the markers were monomorphic and, after the marker were filtered, we were able to use just 245 markers to look for QTLs. Unfortunately she did not find any QTLs due to insufficient marker coverage. Several of the lines had yields similar to Louise, however, indicating that this population may be useful as a source of novel resistance for introgression. Because of COVID, these lines were not planted into the field this year.

Twenty-one additional land Iranian land races were evaluated in the greenhouse, and two showed consistent tolerance in seedling assays (AUS28329 and AUS28714).

#### **Deliverables**

1. Resistance ratings of spring and winter varieties grown in WA
2. Contributed to release of a new soft white winter wheat variety, Devote, with high Fusarium crown rot tolerance as well as other soil borne diseases.
3. Identification of new QTLs for resistance to *F. culmorum*.
4. Better greenhouse and field methodology for screening for Fusarium resistance in greenhouse
5. Identification of several spring and winter wheat germplasm lines that have shown resistance over multiple greenhouse and field screening assays. These lines will be used in recurrent selection breeding to improve combine resistance to Fusarium crown rot with that of other soil borne diseases in both spring and winter wheat.

## Refereed papers

Thompson, Y. A., Paulitz, T. C. and Garland-Campbell, K. A. 2020. Genome-Wide Association Study for Fusarium Crown Rot (*Fusarium culmorum*) Resistance in a Diverse Global Wheat (*Triticum aestivum* L.) Collection. BMC Plant Biology (submitted)

## Theses

Waziri, A. D. 2019. Genetic and metabolic determinants of resistance to Fusarium crown rot in spring wheat. MSc thesis. Washington State University July 2019.

Yvonne Manning. 2018. Identification of Quantitative Trait Loci (QTL) for Resistance to Soil-Borne Pathogens *Fusarium culmorum* and *Heterodera filipjevi* in Wheat (*Triticum aestivum* L). PhD Thesis, Washington State University, Pullman, WA

Swannack, Carmen. 2019. QTL analysis of end-use quality and stripe rust resistance traits in a double haploid soft wheat population. MSc Thesis, WSU, Dec. 2019.

## Popular articles

Paulitz, T. C. and Campbell, K.G. 2019. Fusarium crown rot: Disease is prevalent, persistent, pernicious. Wheat Life, June 2019

**WGC project number:** 3019-3685  
**WGC project title:** Fusarium Crown Rot on Wheat: Prebreeding and Development of Tools for Genetic Disease Management  
**Project PI(s):** M. Pumphrey, K. Garland-Campbell, and T. Paulitz  
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**Project year:** Year 3 2020-2021

Objective	Deliverable	Progress	Timeline	Communication
Objective 1. Screen spring and winter variety trials and breeding lines for resistance in the greenhouse.	Ratings of varieties for <i>Fusarium</i> tolerance in the the WSCIA seed buyers guide and other publications.	For the first time, a <i>Fusarium</i> tolerant variety has been released based on greenhouse screening. This soft white winter wheat, Devote, also has tolerance to strawbreaker foot rot, can be planted deep, and is adapted to low rainfall zones. We continue to screen varieties in the greenhouse, and are still trying to optimize the methods to reduce variability. In 2020, we screened 328 lines, which was a total of 1,312 cones and collected over 20,000 data points from that screening. We experimented with grinding the millet inoculum and quantifying it with dilution planting. The previous method relies on whole millet seed. We also experimented with different rating systems. Rating the number of internodes infected gave the highest correlation with yield loss, the difference in thousand kernal weight between the inoculated and control.	Greenhouse screening will continue with optimized methods in 2021-2022	
Objective 2. Select for QTLs associated with resistance in segregating populations	Resistant sources that can be used for variety development.	A doubled haploid population from Cara/Xerpha was evaluated in the greenhouse. Fifteen lines showed lower disease ratings than Xerpha and Madsen. These lines can be introgressed directly. We also initiated screening of the DNAM population, specifically U6713 and UC6716. This is a population of crosses with <i>A. tauschii</i> , focused on the D genome which contains novel resistance. This population was highly skewed toward resistance.	Because this population is already PNW adapted soft white wheat, the lines with better resistance will be crossed to some of our better <i>Fusarium</i> resistance sources in 2021.	Yvonne, M., Paulitz, T. C. and Campbell, K. G. 2020. Genome-wide association study for <i>Fusarium</i> crown rot in a diverse wheat germplasm. Submitted to BMC Plant Biology. Waziri, A. D. 2019. Genetic and metabolic determinants of resistance to <i>Fusarium</i> crown rot in spring wheat. MSc thesis. Washington State University July 2019. Swannack, Carmen. 2019. QTL analysis of end-use quality and stripe rust resistance traits in a double haploid soft wheat population. MSc Thesis, WSU, Dec. 2019.
Objective 3. Look for new sources of resistance in a new set of synthetic wheat that was developed by CIMMYT in Turkey.	Resistant sources that can be used for variety development.	We have screened a large collection of crosses with the synthetics, with parents from each breeding class crossed to SYNT-IN 15 and 16, the most resistant synthetic lines. These are currently at the BC1F2 stage. The frequency distribution was highly skewed to resistance, with over 150 crosses with a rating of 1. We also rated a population of synthetics crosses with Louise, part of research on <i>Rhizoctonia</i> resistance initiated by Scot Hulbert over 10 years ago. These populations are also highly skewed toward resistance.	Greenhouse screening of backcrosses will continue in 2021-2022.	

