Final Report for Fusarium Grant 2021-2023

 # GR00008054. AWD 3581

Progress Report Year: **final report of three year cycle**

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

Investigators: Kimberly Garland-Campbell, Timothy Paulitz, and Rich Koenig (fund holder)

Cooperators Arron Carter, Mike Pumphrey, Nuan Wen, Ron Sloot and Patricia Demacon.

Executive summary Over the last three years, we have focused on developing a better greenhouse screening system to rate Fusarium resistance. We have optimized temperature, water stress, inoculum quantification and rating system to reduce variability and increase disease pressure. We are presently screening about 500 lines per year for resistance, including all of the Winter and Spring Regional Nurseries, and the advanced lines in the variety testing sites. We have identified several varieties with increased tolerance, including the winter wheat Devote. Secondly, we continue to develop collections of germplasm that are better adapted to PNW conditions, and these are being incorporated into the breeding programs.

Introduction:

Fusarium crown rot is the most widespread and chronic soilborne disease in the Pacific Northwest, causing an average of 10% yield loss and an estimated $ 80 million dollars in losses. There are presently no chemical controls, growers have to rely on cultural controls such as crop rotation or N management, which are not effective. Resistance or tolerance would be the best solution, but there is no major gene resistance present. In addition, the disease is highly affected by environmental conditions, increased with water stress, making screening difficult in the field. That is why we are optimizing greenhouse screening techniques and developing more effective germplasm.

Over the last 3 years, our main objectives have been to continue to identify new and existing sources of resistance that can be used in the WA breeding programs to create tolerant varieties. Finding tolerance or 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. This has been used to screen all the advanced variety testing lines, both winter and spring, as well as the Winter and Spring Regional Nurseries. We are presently screening over 200 varieties/lines per year. But the biggest advance the last three years has been to expand our pool of germplasm collections to include exotic sources that are more amenable to incorporation into adapted germplasm.

Approach:

1). Develop 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 (work of Yvonne Thompson supported by previous cycle). With the PhD research of Nikayla Strauss in this cycle, we developed a better method of quantifying inoculum. Rather than just placing whole millet seed next to the seedling, the inoculum was ground and spread around the surface for a more uniform coverage. In addition, we quantified the strength of the inoculum by serial dilution plating. We diluted the ground inoculum in water, created a suspension, and spread it on agar media to count the number of colonies. Then the total colony forming units in the original inoculum can be calculated. The other advance was in developing a faster more accurate method of phenotyping. We were using a 1-9 rating system, by comparing the appearance to photos corresponding to each disease level. Instead, by counting the number of discolored internodes, we can count faster and get more reproducible results. It is also easier to train rating personnel. We evaluated the methods with a power analysis, and found the new method took less than half the number of replicates to accurately rate the varieties. One way to overcome variation is to increase the number of replicates, but this takes more greenhouse resources.

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 rated because of a lack of disease. It also assumed uniform inoculum in the field. One approach taken by Richard Smiley and others was to apply inoculum at the time of seeding in the field in paired rows. This is very time consuming, and can only be used for evaluating a few varieties. This program has not been continued by his replacement, Christina Hagerty. 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

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 novel and more adapted 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 results. These include

a. A set of facultative synthetic wheat germplasm developed by CIMMYT in Turkey that were crossed with locally adapted varieties. Parent PNW varieties included spring wheats Chet, Ryan, DH11SRW070-14, and winter wheats Selbu, WA8252, Sequoia, and club wheat Cara.

b. Populations developed by backcrossing Iranian landrace AUS28451 to Louise.

c. Doubled haploid population from Cara X Xerpha cross.

d. 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.

Results:

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 *F. pseudograminarum* 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 *Fusarium* inoculum and a better rating system**. 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. For spring wheats, 'AP Coachman', 'JD', 'Ryan', 'Roger', 'CP3530', 'CP3119A', and 'Net Cl+', and several LDRC and WA breeding lines were as resistant as the resistant checks '2-49' and 'Sunco'. Winter wheats are more variable and more testing is needed but better resistance was demonstrated by 'AP-Exceed', 'Rosalyn', and 'TMC M-pire'.

We evaluated the spring and winter wheat variety trials using the greenhouse test. We confirmed our predictions that at least 8 replications were required for stable data.

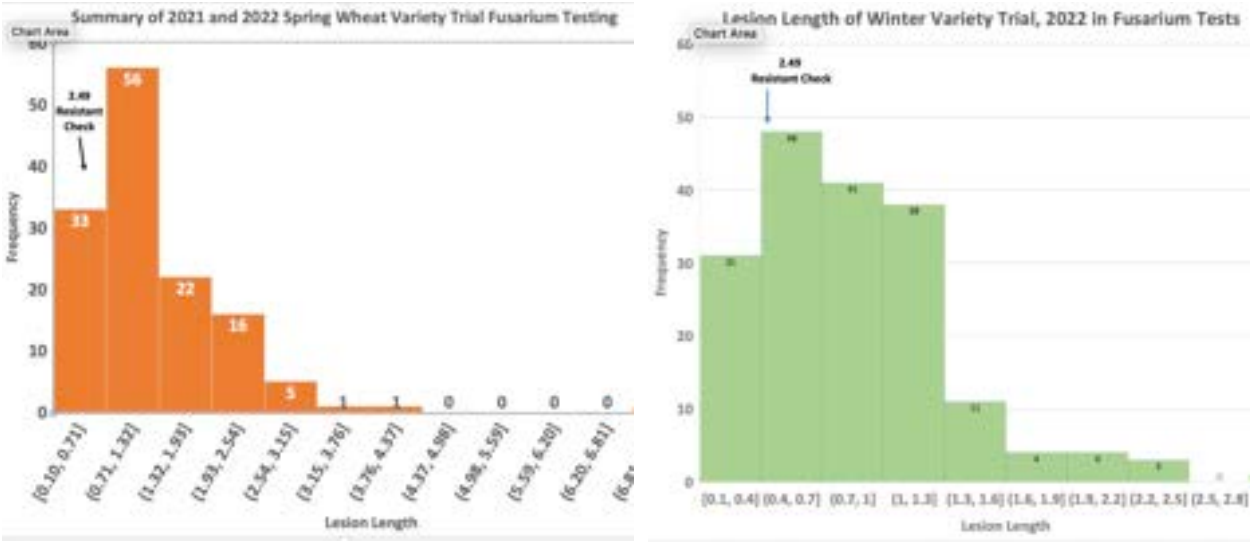


Fig. 1. Distributions of Fusarium resistance in spring and winter wheat variety trials. In both cases, there are cultivars that rated better than our resistant check, the Australian line '2.49'.

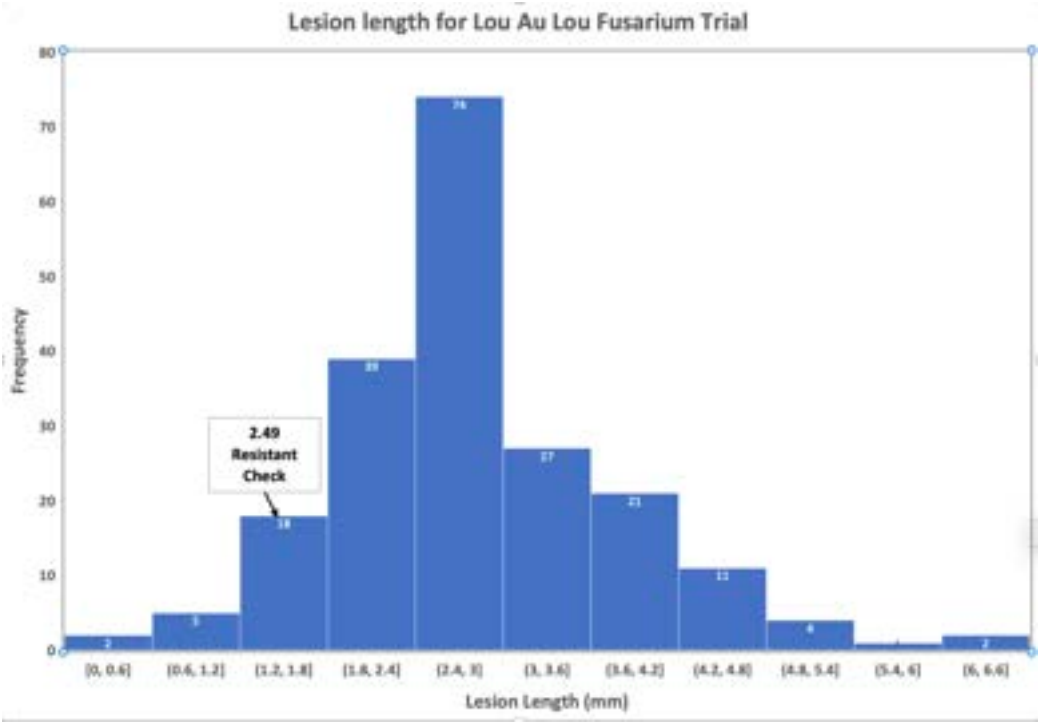
Objective 2. Look for new sources of resistance in a new set of synthetic wheat that was developed by CIMMYT in Turkey and in other collections.

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. The adapted parents included the spring wheats Chet, Ryan, DH11SRW070-14, and the winter wheats Selbu, WA8252, Sequoia, and club wheat Cara. We have increased these populations to advance them from the F2 to the F4 and will be evaluating them in 2024 and 2025.

We developed a doubled haploid population from Cara/Xerpha which was evaluated in the greenhouse. Fifteen lines showed lower disease ratings than Xerpha and Madsen. These lines can be introgressed directly into adapted soft white winter breeding lines. This population has not yet been incorporated into the breeding program.

Another source of resistance is from a DNAM population, specifically from the *A. tasuchii* 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. This population has not yet been evaluated.

We screened the Aus28451/Louise backcross population (Lou Au Lou) and discovered several lines that are more resistant than our resistant check. These will be crossed to our most resistant spring and winter wheat cultivars to combine resistance sources.



Objective 3. Breed for Fusarium crown rot resistance using our greenhouse and field screening systems and marker assisted selection for other important traits for wheat in the Pacific Northwest, (for example: eyespot and stripe rust resistance; grain quality, reduced height, and cold tolerance).

We have used marker assisted selection to select for resistance to strawbreaker foot rot (eyespot) for stripe rust resistance and for end use quality. We have concluded that Fusarium is too complex a disease to select for with marker assisted selection. We will initially use marker assisted selection for other traits, then evaluate for Fusarium resistance.

Impact

We are increasing the Fusarium resistance and tolerance of WA varieties.

Ovcr the last three years, we have

1. Provided 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 soilborne diseases.
3. Developed new exotic sources of resistance that can easily be incorporated into adapted PNW varieties.

4. Optimized better greenhouse and field methodology for screening for Fusarium resistance in greenhouse.
5. Identified 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 and combine resistance to Fusarium crown rot with that of other soilborne 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. Submitted to BMC Plant Biology, not accepted. We are resubmitting this to Crop Science this spring.

Strauss, N., Klarquist, E., Kaya, J., Thompson, Y, Paulitz, T. C. and Garland Campbell, K. 2022. Screening of Winter Wheat for Fusarium Crown Rot in a Controlled Environment. Frontiers in Plant Science (To be submitted)

Theses

Strauss, Nikayla. 2022. Identifying Novel Disease Resistance and Drought Tolerance Genes in a Synthetic NAM Population PhD Thesis, Washington State University

Popular articles

Paulitz, T. C. and Campbell, K.G. 2023. Fusarium Crown Rot of Wheat- It's Everywhere and Persistent Wheat Life, Dec. 2023

Podcasts

WSU Wheat Beat Podcast. Fusarium Crown Rot. Recorded and published December 2023.

WGC project number:
WGC project title: **Fusarium Crown Rot on Wheat: Prebreeding and Development of Tools for Genetic Disease Management**
Project PI(s): **K. Garland-Campbell, T. Paulitz and R. Koenig**
Project initiation date: **7/1/2021**
Project year: **Year 3 2023-2024**

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 *Fusarium* tolerance in the the WSCIA seed buyers guide and other publications.	In 2023 we screened the Lou-Au Lou population which includes genetic resistance derived from an Iranian landrace. We planted the 2024 WA winter wheat variety trials for screening. Growth chamber space limitations reduced the number of lines that we can screen each year, so we have changed the procedure slightly to use additional chambers and greenhouses to screen the 2024 WA winter wheat trials. After two years of screening spring variety trials, we can say that several cultivars including 'AP Coachman', 'JD', 'Ryan', 'Roger', 'CP3530', 'CP3119A', and 'Net Cl+', and several LDRC and WA breeding lines are as resistant as the resistant checks '2-49' and 'Sunco'. Winter wheats are more variable and more testing is needed but better resistance is demonstrated by 'AP-Exceed', 'Rosalyn', and 'TMC M-pire'.	Greenhouse screening will continue with optimized methods in 2024-2025	Paulitz, T. C. and Campbell, 2023. Fusarium Crown Rot of Wheat- It's Everywhere and Persistent. Wheat Life, Dec. 2023. Fusarium Crown Rot- Wheat Beat Podcast, recorded Dec. 2023
Objective 2. Look for new sources of resistance in a new set of synthetic wheat that was developed by CIMMYT in Turkey and in other collections	Resistant sources that can be used for variety development.	Crosses have been made with soft white winter wheat lines such as NW Tandem, that are exhibiting more resistance to FCR. We will select progeny during early generation increase using our improved screening system, followed by marker assisted selection for resistance to other soil borne diseases such as strawbreaker foot rot.	Crosses will be evaluated, advanced, and intercrossed in 2024 and 2025.	Paulitz, T. C. and Campbell, 2023. Fusarium Crown Rot of Wheat- It's Everywhere and Persistent. Wheat Life, Dec. 2023. Fusarium Crown Rot- Wheat Beat Podcast, recorded Dec. 2023
Objective 3. Breed for Fusarium crown rot resistance using our greenhouse and field screening systems and marker assisted selection for other important traits for wheat in the Pacific Northwest, (for example: eyespot and stripe rust resistance; grain quality, reduced height, and cold tolerance)	Resistant sources that can be used for variety development.	We did run a field trial at Lind this summer but weren't able to rate it due to the extremely dry and hot spring and a problem with the irrigation system. This was planted into durum wheat the previous year to build up inoculum. We've corrected the problem and plan to plant a spring trial at Lind this year as well as a winter trial in fall 2024.	Greenhouse screening of backcrosses will continue in 2024-2025.	Paulitz, T. C. and Campbell, 2023. Fusarium Crown Rot of Wheat- It's Everywhere and Persistent. Wheat Life, Dec. 2023. Fusarium Crown Rot- Wheat Beat Podcast, recorded Dec. 2023