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***Washington State University
Wheat and Barley Research
Progress Reports***



2020-2021 Fiscal Year

2020-21 WSU Wheat & Barley Research Progress Reports to the Washington Grain Commission

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Washington Grain Commission Wheat and Barley Research Final Report

Project #: 3019-3009

Progress Report Year: 3 of 3

Title: Improving Barley Varieties for Feed, Food and Malt

Cooperators: Deven See, Xianming Chen, Max Wood

Executive Summary: During my first field season as the WSU barley breeder and PI of this grant we made excellent progress considering the difficulties imposed by COVID-19. The Washington Grains Commission (WGC) funds were directed at enhancing the WSU spring malt barley breeding program and establishing a winter malt barley breeding program. The major goal of both spring and winter classes of malt barley is the development of varieties that make the American Malting Barley Association (AMBA) recommended list.

To quickly achieve greater efficiency in the WSU malt barley breeding programs we needed to add or upgraded equipment, instrumentation and technologies. The most important addition to instrumentation and capabilities included the development of the state-of-the-art WSU Malt Quality Lab (WMQL) that was funded by the WGC. This facility is projected to be analyzing 2019 field season material in February, 2020, which will provide much greater efficiency when selecting early and late generation materials for malt quality. To aid the rapid early generation selection we also equipped the WSU barley breeding and molecular lab with the capability to run PCR genotype-by-sequencing libraries for amplicon sequencing and added the computational power to run the bioinformatics analysis. In collaboration with Dr. Deven Cee's USDA cereal genotyping lab we developed a panel of 100 DNA markers associated with malt quality traits initially focused on malt extract, low protein, and low beta-glucan. This will allow us to utilize marker assisted selection (MAS) and genomic selection (GS) strategies to further our capability to make early generation quality predictions and select progeny for malt quality characteristics. Thus, the early generation spring head rows (~10,000/year), advanced single rep yield trials (~500/year) and multi rep yield trials (~50/year) will be enriched for malt quality so we can concentrate on selecting for farmer traits including yield, height, standability and disease resistance in the field utilizing traditional selection strategies. This capability will also be important in the winter malt barley selection processes as we begin advancing these materials in the program which will double our materials in the field each season.

To aid in the efficient later generation selection the major piece of equipment and upgrade was our new "Wintersteiger classic plus" plot combine with the Harvest Master H2-Classic weighing system. The new combine provides us with real time yield, test weight and moisture data, saving time and funding previously dedicated to threshing and analysis. This will allow for greater field capacity of later generation lines, which will be very important as we add winter field nurseries and trials to the breeding program. This will provide much more efficient use of the WGC funds and will allow for greater capacity to analyze later generation material that have already been screened for quality in the WMQL and via marker assisted selection of both the advanced spring malt barley lines and future advanced winter barley lines.

Impact: In the 1.5 years as the PI on this WGC barley breeding grant the WSU barley program has continued advancing feed, food and malting populations and lines in the field at Spillman Farm and greenhouses on the WSU campus. In the last year we started shifting the focus of the WSU breeding program mainly to malt barley variety development including both spring and winter classes of barley. In my first field season and PI on this grant we made over 200 new crosses focused on malt quality and targeted agronomic traits and disease resistance, screened approximately 12,000 single head plots and selected nearly 400 candidate malt barley progeny lines for single rep yield trials for the 2021 field season. All lines have been harvested and processing to collect data for selection and advancement of elite material. In the 2020-2021 greenhouse the second spring malting barley crossing blocks were planted with selected high quality malting parental lines and high yielding adapted PNW germplasm selected with increased malt quality. Ten elite crosses were picked and are currently being advanced to plant an additional 10,000 F₃₋₄ head rows in the field for evaluation in the 2021 field season.

Winter malting and feed barley lines were also selected and planted to begin crossing for the WSU winter barley breeding program that was initiated in the Fall of 2020. In Fall 2019 we planted over 600 elite and advanced winter malting lines provided by Dr. Pat Hayes, the Oregon State University winter malt barley breeder, to begin the process of identifying parental materials for the WSU breeding program that perform well at Spillman farm in terms of both quality and yield. We are also continuing to evaluate and advance IMI-tolerance in multiple classes of selected elite barley lines.

To better utilize molecular marker selection current parental lines and breeding material from the WSU breeding program are being evaluated by genotyping with malt quality molecular markers. In collaboration with Dr. Deven See we are developing a new amplicon sequencing PCR targeted Genotype-By-Sequencing (PCR-GBS) platform adapted to his Illumina sequencer. This was necessary because the USDA-ARS cereal genotyping lab quit supporting their Ion Torrent system for which we had designed our previous panels described in the previous WGC grant and report. We are currently utilizing this platform to screen 150 two-rowed malting parental lines and F₁ progeny to validate the system and once this training set is validated will begin screening our early generation selections with the markers associated with low grain protein, increasing malt extract, and reduced wort β -glucan. Once a training population has been selected and genotyped from the WSU 2020 field lines with malting data collected by our WMQL we will test prediction accuracy. Then we can begin utilizing the marker panel for future marker assisted selection and genomic selection.

We collected a virulent population of *Puccinia graminis* f. sp. *tritici* (*Pgt*) from three Washington barley fields and from the alternate hosts Mahonia and barberry from locations in Spokane, Colfax, and Pullman, WA and Moscow and Potlatch, ID . We generated nearly 200 hundred single pustule isolates and inoculated them onto lines containing the only two effective barley stem rust resistance genes, *Rpg1* and *rpg4/Rpg5*. We identified 10 isolates that are highly virulent on both *Rpg1* and *rpg4/Rpg5*. This type of virulence has never been reported as the combination of the two genes has always provided resistance to all known isolates collected worldwide. These data show that this *Pgt* population contains the most virulence isolates in the world in regards to barley virulence and pose a potential issue to barley and wheat production in Washington and the region. We conducted phenotypic and genotypic screening of the World Barley Core Collection (WBCC) with the most virulent isolate Lsp21 and utilized association

mapping to identify new sources of resistance on barley chromosomes 2H and 5H that are effective against the Washington State *Pgt* virulent isolates. In our 2020 field barley crossing block we hybridized lines containing these novel resistance sources with malt barley parental lines and are using a backcrossing scheme to introduce these resistances into our breeding materials.

As we further optimize and streamlining our selection processes including high throughput genotyping and phenotyping procedures this infrastructure investment early in the development of my program will have a measurable impact on optimizing the return on the WGC's funding investment. This investment in time and resources now will expedite the development of AMBA recommended malt barley varieties in the future. As we move the breeding program forward these investments will have measurable impact on the ability to make more precise selections from intensive hybridization and screening of larger numbers of recombinant individuals. Our major focus will be on fixing malt quality in the program while increasing agronomic traits such water use efficiency, disease resistance and ultimately yield.

The most measurable impact(s) this project had in the most recent funding cycle is the transformation of the WSU barley breeding program into a spring and winter malt barley breeding program. This includes the addition of important infrastructure and support instrumentation that will allow us to quickly shift gears towards the goal of premium AMBA recommended spring and winter malt barley varieties. Although, I may not report on the development and release of these varieties in the short term, the goal of my career at WSU is to transform the program into a top malt barley breeding program and see a major increase in barley acreage to feed the domestic supply of quality malting barley as well as international markets. Considering that this is my first year as the barley breeder at WSU, I believe we have made considerable progress towards achieving our long term goals.

Outputs and Outcomes:

Objective	Deliverable	Progress	Timeline	Communication
1	Release of the malting barley cultivar Palmer suited to brewing and distilling	Several of the advanced malting barley breeding lines have performed well in malting end use quality tests and agronomically in Variety Testing. A second WSU malting line should be released in the coming year	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts

2	Release of a second IMI-tolerant barley variety with high yield and excellent disease resistance to complement Survivor in the coming two years.	We have thousands of known IMI-tolerant barley lines in our breeding pipeline. These have and will continue to undergo greenhouse and field trials as well as multi-location yield trials to identify the superior breeding lines available.	2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
3	Hulless, waxy food barley variety release to support non-waxy high beta glucan varieties Havener and Meg's Song	Our hulless, high Beta-glucan breeding lines are performing well in the advanced breeder trials and will be included in variety testing trials	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts
4	One new two-row feed spring barley variety released with superior yield disease resistance, protein and agronomic characteristics	Two two-row spring varieties were released in 2013. The elite breeding lines are showing excellent promise to exceed these and other existing varieties.	2021-2022	Talks and presentations at field days; distribution of informative variety rack cards; Wheat Life articles; Pod Casts

Communications continued

Publications are listed here as the block above was too small.

Peer-Reviewed Publications/Preprints (16 total) and Book Chapters (3 total):

Published, Accepted or Submitted from December 1, 2019 – December 31, 2020

Peer-Reviewed Publications/Preprints:

1. Tamang, P., Richards, J.K., Solanki, S., Ameen, G., Sharma Poudel, R., Deka, P., Effertz, K., Gross, T., Hegstad, J., Rehman, S., Bezbaruah, A., Li, X., Horsley, R.D., Friesen, T.L. and **Brueggeman, R.S. (2020)** The barley *HvWRKY6* transcription factor is required for resistance against *Pyrenophora teres* f. *teres*. *Frontiers in Genetics* 11:601500. doi:10.3389/fgene.2020.601500
2. Restrepo-Montoya, D., **Brueggeman, R.S.**, McClean, P., and Osorno, J.M. (2020) Computational identification of receptor-like kinases “RLK” and receptor-like proteins “RLP” in legumes. *BMC Genomics* 21(1) DOI: 10.1186/s12864-020-6844-z.

3. Solanki, S., Ameen, G., Zhao, J., Flatten, J., Borowicz, P., and **Brueggeman, R.S. (2020)** Visualization of spatial gene expression in plants by modified RNAscope fluorescent in situ hybridization. *Plant Methods* 16(1) DOI: 10.1186/s13007-020-00614-4
4. Sanyal, D., Solanki, S., Ameen, G., **Brueggeman, R.S.**, and Chatterjee, A. (2020) Understanding the expression dynamics of symbiont rhizobial nifH and nitrogen assimilatory NR and GS genes in dry bean (*Phaseolus vulgaris* L.) genotypes at various growth stages. *Legume Science* DOI: 10.1002/leg3.26.
5. Gyawali, S., Reda, A., Verma, R.P.S., **Brueggeman, R.S.**, Rehman, S., Belqadi, L., Arbaoui, M., Tamang, P., and Singh, M., (2020) Seedling and adult stage resistance to net form of net blotch (NFB) in spring barley and stability of adult stage resistance to NFB in Morocco. *Journal of Phytopathology* 168(39) DOI: [10.1111/jph.12887](https://doi.org/10.1111/jph.12887).
6. Aoun, M., Kolmer, J.A., Breiland, M., Richards, J., **Brueggeman, R.S.**, Szabo, L.J., and Acevedo, M. (2020) Genotyping-by-Sequencing for the Study of Genetic Diversity in *Puccinia triticina*. *Plant Disease* 104(3) DOI:10.1094/PDIS-09-19-1890-RE.
7. Ameen, G., Solanki, S. Drader, T., Sagar-Bittara L., Steffenson, B.J., Kleinhofs, A., Vogiatzis, C., and **Robert S. Brueggeman (2020)** A wall-associated kinase gene is the spot blotch susceptibility gene *rsc5* in barley. *bioRxiv* doi:<https://doi.org/10.1101/2020.04.13.040238>
8. Jin, Z., Solanki, S., Ameen, G., Gross, T., Sharma Poudel, R., Borowicz, P., **Brueggeman, R.S.**, and Schwarz, P. (2020) Localization of hyphal growth associated with mycotoxin production during the malting of Fusarium head blight infected grains. *bioRxiv* doi:<https://doi.org/10.1101/2020.06.06.126979>.
9. Ameen, G., Solanki, S. Drader, T., Sagar-Bittara L., Steffenson, B.J., Kleinhofs, A., Vogiatzis, C., and **Robert S. Brueggeman (2020)** A wall-associated kinase gene is the spot blotch susceptibility gene *rsc5* in barley. *Proceedings of the National Academy of Sciences, USA*, (Submitted).
10. Ameen G., Bittara, L., Ameen G., Richards, J., Tamang, P., Solanki, S., Friesen, T.L, and **Brueggeman, R.S. (2020)** The *Nec3* gene is a putative negative regulator of pathogen induced programmed cell death in barley. *New Phytologist* (Submitted).
11. Zhang, Z, Running, K.L.D., Seneviratne, S., Peters-Haugrud, A. R., Szabo-Hever, A., Shi, G., Luo, M.-C., **Brueggeman, R.S.**, Xu, S.S., Friesen, T.L., and Faris J.D. (2020) A protein kinase-major sperm protein gene hijacked by a necrotrophic fungal pathogen triggers disease susceptibility in wheat. *The Plant Journal* (Submitted).
12. Capo-chich, L., Eldridge, S., Elakhdar, A., Kubo, T., Kamamaru, T., **Brueggeman, R.S.**, and Anyla, A.O. (2020) QTL Mapping and Phenotypic Variation for Seedling Vigour Traits in Barley (*Hordeum vulgare* L.). *Frontiers in Plant Science* (Submitted).
13. Jin, Z., Solanki, S., Ameen, G., Gross, T., Sharma Poudel, R., Borowicz, P., **Brueggeman, R.S.**, and Schwarz, P. (2020) Localization of hyphal growth associated with mycotoxin production during the malting of Fusarium head blight infected grains. *Molecular Plant Microbe Interactions* (Submitted)
14. Sharma Poudel, R., Richards, J., Shestha, S., Solanki, S., and **Brueggeman, R.S. (2019)** Transcriptome-wide association study identifies putative elicitors/suppressor of *Puccinia graminis* f. sp. *tritici* that modulate barley *rpg4*-mediated stem rust resistance. *BMC Genomics* 20(1) doi: 10.1186/s12864-019-6368-7.
15. Clare, S., Wyatt, N., **Brueggeman, R.S.** and Friesen, T. (2019) Research advances in the *Pyrenophora teres*–barley interaction. *Molecular Plant Pathology* 21(2) DOI:10.1111/mp.12896.
16. Haque, M.E., Bloomquist, M., Bhuiyan, M.Z.R., Gross, T., Hakk, P., Leng, Y., Liu, Y., Zhong, S., **Brueggeman, R.S.** Sharma Poudel, R., and Kahn, M. (2019) First Report of Alternaria Leaf Spot Caused by *Alternaria tenuissima* on Sugar Beet (*Beta vulgaris*) in Minnesota, USA. *Plant Disease* <https://doi.org/10.1094/PDIS-03-19-0603-PDN>.

Book Chapters:

1. Solanki, S., Ameen, G., Sanyal, D., Jain, S., Elakhdar, A., Lall, S., Chittem, K., Brueggeman, L., Kumar, A., and **Brueggeman, R.S. (2020)** Friends and Foes: Phyto-Microbial Interactions in Molecular Perspective. In: Kumar M., Kumar V., Prasad R. (eds) Phyto-Microbiome in Stress Regulation. Environmental and Microbial Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-15-2576-6_5.
2. **Brueggeman, R.**, Solanki, S., Ameen, G., Effertz, K., Sharma Poudel, R., and Karakaya, A., (2020) Fungal Diseases Affecting Barley. In: Fox, G.P. and Li, C. (eds) [Achieving sustainable cultivation of barley](#). Burleigh Dodds Science Publishing, UK. DOI: 10.19103/AS.2019.0060.16.
3. Effertz, K.M., Clare, S.J., Harkins, S.M., and **Brueggeman, R.S. (2020)** Understanding plant-pathogen interactions in net blotch infection of cereals. In Oliver, R. (ed) Achieving durable resistance in cereals. Burleigh Dodds Science Publishing, UK (In Press)

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

Project #: 3019-3155

Progress Report Year: 2 of 3

Title: Weed Management in Wheat

Researcher(s): Ian C. Burke and Drew J. Lyon

Executive summary: Weed control is one of the major challenges facing wheat growers in the PNW. To address this problem, the Weed Science Program conducts a multi-disciplinary field, greenhouse, and laboratory research project to address the critical issues that Washington wheat growers face. One aspect of this work is the evaluation of herbicides, both registered and nonregistered, for crop tolerance and weed control in wheat production systems. This work is often, but not always, conducted in partnership with agricultural chemical companies. These field studies allow us to make better recommendations to growers, and they provides us the opportunity to work with the various companies to better refine their labels for the benefit of Washington wheat growers. The results from these studies are summarized in the WSU Weed Control Report, which is shared with the Washington Grain Commission and posted on the WSU Extension Small Grains website annually. The Weed Science Program continues to look at the biology and ecology of troublesome weeds including downy brome, Russian-thistle, and mayweed chamomile.

Impact: The WSU Weed Science Program impacts wheat and barley production in Washington and the Pacific Northwest by producing timely, accurate, non-biased weed control and weed biology information. That information is most commonly extended to stakeholders in the form of presentations, extension publications, news releases, and the Internet (wsu.smallgrains.edu). In terms of value, herbicide inputs are typically among costliest a grower faces, and using the most economical and effective treatment will improve the net income and long term sustainability of any operation

- The project continues to generate data and local insights for various agrichemical companies to assist them in labeling their new herbicide products for weed control in wheat. We have been working with bicylopyrone, a new broadleaf herbicide from Syngenta, as well as new herbicides from Corteva, Bayer, FMC, and old herbicides from Albaugh and Gowan.
- A number of grower driven projects were continued in the new cycle, including management of rush skeletonweed and other troublesome weeds in fallow, management of scouringrushes in wheat, use of weed sensing sprayers in fallow, spring wheat preemergence herbicides for Italian ryegrass control, and management of brome species with preemergence herbicides.
- Extending the outputs of the project was a challenge during the pandemic. Dr. Lyon continued to publish extension output in the form of webcasts and extension bulletins based on our research, and several presentations were created in active field trials. We look forward to resuming our active extension grower engagement in 2021.

WGC project number: 3019-3155
WGC project title: Weed Management in Wheat
Project PI(s): Ian C. Burke and Drew J. Lyon
Project initiation date: July 1, 2019
Project year: 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
Evaluate herbicides	Efficacy and crop injury data to support use recommendations, new labels, and label changes to benefit WA small grain growers.	The WSU Weed Control Report was published annually and distributed to the Washington Grain Commission, County Extension Educators in eastern Washington, and sponsoring chemical companies. The published studies are posted on the WSU Extension smallgrains website and discussed at winter Extension meetings.	Annually, in time for winter meetings.	Annual weed control report; articles in Wheat Life, trade magazines and/or posted to WSU smallgrains website; field days; winter Extension meetings; decision support system tools. The Small Grains website now host an outlet for our efficacy results see https://herbicideefficacy.cahnrs.wsu.edu/
		A 5-year field study was initiated in 2017 near Omak to look at how frequently Finesse and Amber herbicides must be used to maintain control of smooth scouringrush in winter wheat-fallow production systems. Two 6-year field studies were initiated in 2019 in the intermediate rainfall zone (Edwall and Steptoe) to determine the same thing in winter wheat-spring wheat fallow production systems. Studies were also initiated in 2019 and 2020 to look at the impact of various surfactants on the efficacy of glyphosate for smooth scouringrush control in fallow.	The Omak study will conclude with fallow stem counts made in the summer of 2021. The results from this study will be reported through extension outlets, but journal publication will have to wait until the completion in 2025 of the two 6-year studies from the intermediate rainfall zone. We will continue the glyphosate plus surfactant studies in 2021 with the expectation of submitting a manuscript for publication in 2022.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles
		Field studies were completed in 2020 near Lacrosse and Hay addressing control of rush skeletonweed in fallow. A manuscript on this work will be submitted for publication in 2021. Field studies comparing various rates of picloram (Tordon 22K) applied broadcast or with a weed-sensing sprayer for the control of rush skeletonweed in fallow were initiated in 2019 and 2020 near Lacrosse and Hay.	The fallow study was completed in 2020. We will submit a manuscript for publication in 2021. The picloram study should be completed after the 2022 wheat harvest.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles
		Multiple field studies were conducted in association with agrichemical companies to investigate efficacy and crop tolerance to a range of grass and broadleaf weed control products. These studies allow us to evaluate new chemistries or new uses of old chemistries and also help us modify company labels to better suit our region.	Field studies will be conducted every year during this project.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles

		A WEED-It sensor sprayer system was acquired in the spring of 2018 using Camp Endowment funds, and multiple experiments were initiated to evaluate efficacy of various fallow herbicides using the sensor system.	Additional trials were installed in the spring of 2021. New trials will be designed based on the trial experiments to evaluate the sensor sprayer when used season long in fallow as well as in crop, and emphasize economics and use of alternate herbicide modes of action.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles
Evaluate weed biology & ecology	Weed biology and ecology to aid in the design of effective and economic control strategies for troublesome weeds in WA small grain crops; decision support system database development.	Common garden and greenhouse experiments using the downy brome PNW core collection have started to identify variation in flowering time. Flowering time genes strongly coordinate with vernalization genes to regulate when flowering occurs in downy brome. In previous work, vernalization regulated if flowering occurred, but it was evident that, when placed in different environments, flowering time was plastic.	A large downy brome panel was phenotyped for flowering time. Initial analyses indicate that flowering time is highly heritable, unlike growth or tillering. Highly heritable traits may facilitate ecological approaches to management of downy brome by field, and indicates that movement between fields should be minimized.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles.
		We have identified a new and potentially troublesome pest in the inland Pacific Northwest. Discovered while conducting field trials near Asotin and Ewan, sterile brome does not appear to respond to postemergence herbicides. The weed has a very similar appearance to downy brome except while flowering, and thus is usually identified as downy brome.	We are preparing an identification guide for <i>Bromus</i> species and will produce an extension bulletin in spring 2021 - the pandemic derailed plans to produce a document in 2020.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles.
Evaluate cultural & mechanical management	Data to support recommendations for integrated weed management systems to control troublesome weeds in WA small grains.	As part of our work to understand seed dormancy in downy brome, we have discovered that gibberillic acid can be used to stimulate germination in the field. Current work has focused on identification of the duration of the effect as well as on additional weed species that may respond to such an input. Recent greenhouse work indicates that soil temperature may play a role in the effect we have observed.	Field studies will be conducted every year during this project.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles.
		We conducted field studies to ascertain seed shattering in Italian ryegrass by harvest time in winter and spring wheat. In 2020, we initiated a field study to evaluate chaff lining for the management of Italian ryegrass in winter wheat.	Italian ryegrass samples collected in 2020 will be evaluated for seed shatter in the winter of 2021. These data will be added to previously collected data and a manuscript will be prepared for journal submission by the end of 2021. Chaff lining experiments will be repeated in 2021.	Annual weed control report, extension publications, extension meetings and field days, and refereed journal articles.

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

Project #: 3193

Progress Report Year: 2 of 3

Title: Field Breeding Hard White and Red Winter Wheat

Investigator/Cooperators: AH Carter, KG Campbell, XM Chen, CF Morris, TD Murray

Executive summary: A new hard red winter wheat lines was released in 2020. This line was named Balance (WA8248), and was released mainly due to performance and interest from the seed industry in Montana. This line has been tested for production in Washington, but it was found that Scorpio (released in 2019) had better performance. Due to its performance in Montana, we decided to release the line rather than discard it from the program. Balance was released mainly because of its resistance to stripe rust, high grain yield and grain protein concentration in Montana, and tolerance to low pH soils, an issue becoming more of a problem in Montana. Recent data suggested that Scorpio has tolerance to Hessian fly, furthering interest in this line. In 2020 we tested multiple other hard red winter lines. Another line which is being considered for release is WA8209, and cultivar which has shown very high grain yield in higher rainfall zones of the state. WA8310 is a hard red winter wheat cultivar targeted to the lower rainfall production areas. This line is a semi-dwarf line, but still shows excellent emergence from deep planting and in dry soils. WA8310 has high yield potential and also maintains a higher grain protein content than other lines at a similar yield potential. In addition to these two lines, WA8318 CL+ (a hard red winter wheat line with 2-gene resistance to imazamox) is being tested. This line is targeted to the low and intermediate rainfall zones of the state, and is targeted to replace acres of SY Clearstone CL2. We continue to watch the hard red market to determine the interest of this line in commercial production. Continued emphasis has been placed on selecting breeding lines with superior quality and disease resistance. We also have a strong interest in developing hard lines with excellent emergence capabilities, and continually screen material to this end. Efforts have been initiated and are ongoing to develop hard cultivars with herbicide tolerance (Clearfield and CoAXium systems), snow mold tolerance, and aluminum tolerance. We maintain about 10% of the hard material as hard white and apply heavy selection pressure to ensure adapted material is advanced. Some of these hard white lines have been tested under irrigation in Southern Idaho and have performed very well. There is interest to release these lines for production under irrigation in Idaho. Our next main target is to develop hard red cultivars with herbicide resistance.

Impact: Sequoia replaced many of the Farnum acres in the state due to its excellent emergence capability and high yield potential under low rainfall and deep planting conditions. Although grown on limited acres, we continue to develop lines with excellent emergence for those regions which need this trait to reduce risk to planting failures under deep planting conditions when moisture is limited. Scorpio is a recent WSU hard red cultivar targeted to high rainfall conditions and will provide growers with a high yielding line with good disease resistance, aluminum tolerance, and Hessian fly tolerance, adapted to PNW growing conditions. Current and future hard red and white lines will continue to lead to a sustainable production of hard wheat in the PNW.

WGC project number: 3193
WGC project title: Development of hard red and white winter wheat
Project PI(s): AH Carter
Project initiation date: July 1, 2009
Project year: 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
Develop hard red and white winter wheat cultivars	New cultivars released for production in WA	In 2019 we released Scorpio, which combines high yield, good protein content, stripe rust resistance, low pH soil tolerance, and Hessian fly tolerance in one line. Seed of Scorpio is being increased by the seed industry and should be available in 2021. The line Balance was released in 2020 for production in Montana, as this line shows high yield and very good protein content, and has low pH soil tolerance. Balance is also available to growers in Washington, but testing suggests Scorpio would be the better selection for Washington. We had over 2,500 plots and 12,000 rows of hard material under evaluation at various stages of the breeding process for 2020. Some hard white winter lines have been submitted for testing in Southern Idaho and have had very good performance under irrigated conditions. These continue to be evaluated for release potential. Focus has also been on developing lines with herbicide tolerance.	Each year we evaluate germplasm at each stage of the breeding process. Each year lines are entered into statewide testing for final release consideration. A cultivar is released, on average, every two years.	Progress is reported through field days, grower meetings, commission reports, popular press, and peer-reviewed manuscripts, and through the annual progress reports
	Agronomic traits	Field trials and agronomic data was conducted and collected at 15 locations in 2020. This includes emergence, winter survivability, heading date, test weight, plant height, and grain yield. Our Kahlotus, Lind, and Ritzville trials gave a very good screen for emergence potential. Our snow mold locations gave a good rating of snow mold tolerance. All other locations had very good stand establishment and we are looking forward to a good year of screening the germplasm.	Evaluation is done annually at multiple locations across the state.	In 2020 we communicated results of this project through the following venues: 14 peer-reviewed publications; 3 virtual field day recordings; 6 field day abstracts; 12 poster presentations; 1 popular press interviews; 3 podcasts; 1 grower meeting presentations; and 3 seed dealer presentations;
	Biotic and Abiotic stress resistance	Lines were screened for snow mold, stripe rust, eyespot foot rot, nematodes, Cephalosporium stripe, SBWMV, Hessian fly, and aluminum tolerance.	Evaluation is done annually at multiple locations across the state.	

	End-use quality	All breeding lines with acceptable agronomic performance in plots were submitted to the quality lab. Those with acceptable milling characteristics were advanced to baking trials. Data should be back in mid-2021. Lines with inferior performance will be discarded from advancement. We screened nearly 1,200 early generation lines for end-use quality in 2020.	Each year, all head rows are evaluated for end-use quality and lines predicted to have superior quality advanced. Each yield trial is submitted for quality evaluations and those with high performance are advanced in the breeding process.	
	Herbicide resistance	Trials were conducted in Lind, Walla Walla, and Pullman for herbicide resistance. The hard red material had a lower priority for development when we started compared to the soft white germplasm, but we now have multiple populations and advanced lines being tested. Crossing has been initiated to incorporate novel herbicide resistance into hard red lines.	Evaluation is done annually at multiple locations across the state	We have entered one hard red line into variety testing with 2-gene resistance to imazamox
Field test adapted germplasm with novel genes introgressed for essential traits	Incorporation of novel genes into adapter germplasm for evaluation under WA environments			Progress is reported through field days, grower meetings, commission reports, popular press, and peer-reviewed manuscripts, and through the annual progress reports
	Rht genes	Populations have been developed and are under field evaluation for Rht1, 2, and 8.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Stripe rust genes	Multiple different stripe rust resistance genes have been introgressed into our germplasm which are under evaluation in Mount Vernon, Central Ferry, and Pullman.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Foot rot genes	Pch1 has been selected for and is under evaluation in field trials in Pullman.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	SBWMV	Crosses are initiated and being evaluated for resistance to SBWMV, mainly first through marker analysis and then under field trials in Walla Walla.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Herbicide tolerance	Hard red lines with herbicide tolerance are being developed in both the Clearfield and CoAXium systems. Populations are first screened in the greenhouse, then transitioned to field testing.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Hessian fly tolerance	With the identification that Scorpio was tolerant to Hessian fly, we have been able to go into our crossing block and find crosses and populations with Scorpio as a parent to begin making selection. These lines will be screened at the University of Idaho as part of the Hessian fly proposal.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS. Screening will be done through project #3674	

	GPC-B1 and Bx7oe	These two genes have been incorporated into many hard breeding lines. These are being tested for agronomic performance in the field. Some lines have already been returned to the breeding program as parents for additional crosses.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
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**Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports**

Project #: 5195

Progress Report Year: 3 of 3

Title: Use of biotechnology for wheat improvement

Investigator/Cooperators: AH Carter, KG Campbell, M Pumphrey, D See

Executive summary: In 2020 we continued our effort to advance breeding lines as quickly and efficiently as possible by employing both molecular marker analysis and doubled-haploid technology. The traits of main focus for marker-assisted selection are foot rot resistance, stripe rust resistance, herbicide tolerance, and end-use quality. All of these traits are already in established breeding lines and have very good markers to track them. Additional traits include aluminum tolerance, SBWMV, dwarfing genes, low PPO, Fusarium head blight, Hessian fly, and nematode resistance. While we have established some breeding lines with these traits, we are working to increase the number of lines carrying these traits, using markers to track their presence. Over 10,000 data points were collected on 200 populations to confirm presence of desired genes, which was a decrease from previous years given the limitations we had accessing our campus facilities. All lines which go through marker testing are then transferred to field testing to confirm the expected phenotype is expressing. Markers were also used to screen all advanced breeding lines to identify presence of known genes. This information was used for selection and advancement purposes (in conjunction with field data) as well as for selecting lines which should be cross-hybridized to create future populations. Our genomic selection efforts are proceeding and we have completed our fifth year of phenotypic evaluations in the field and genotyping. Data is being used to validate selection models for multiple traits. In the greenhouse, we made approximately 650 crosses consisting mainly of soft white and hard red germplasm. We also started a large crossing block to begin widely incorporating new traits of interest, mainly herbicide resistance and pest resistance traits. These are being advanced to the F1 generation, and then divided between our MAS protocol and generational advancement. We planted ~2,000 DH plants in the field in 2020 for evaluation. The remaining DH lines are undergoing increase in the greenhouse and will have a similar number ready for planting in 2021. Our screening process has been slightly altered to allow for marker selection after some field selection is completed.

Impact: This project covers all market classes and rainfall zones in the state of Washington, with about 70% of the effort on soft white crosses. This work will improve end-use quality, genetic resistance to pests and diseases, and agronomic adaptability and stability of released cultivars. All cultivars released (Otto, Puma, Jasper, Sequoia, Devote, Stingray CL+, Scorpio, Purl, Piranha CL+, Sockeye CL+) have benefited through this project by incorporation of disease and end-use quality genes. Released lines have gained popularity and are growing in demand due to the gene combinations they were selected for. The breeding program as a whole has become more efficient in the selection process, and more focus is placed on field evaluations since known genes are already confirmed to be present in the breeding lines. Continued success will be measured by increases in acreage of these lines as well as enhanced cultivar release through DH production, marker-assisted, and genomic selection.

WGC project number: 5195
WGC project title: Use of biotechnology for wheat improvement
Project PI(s): AH Carter
Project initiation date: July 1, 2009
Project year: 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
Marker-assisted selection				Results are presented through annual progress reports, the research review, field tours, and grower meetings
	Foot rot resistant lines	In 2020, all lines under field testing were screened for both Pch1 and Pch2 markers. This information was used to assist selection of lines for further testing under the field program. As more lines are selected for advancement and recycled in the breeding program for new cross-hybridizations, fewer populations will be segregating for this trait.	Each year new crosses are made to Pch1 and Pch2 containing lines. These are subsequently developed, screened, and advanced to state-wide yield trials. At any given time, lines are in every stage of development	In 2020 we communicated results of this project through the following venues: 14 peer-reviewed publications; 3 virtual field day recordings; 6 field day abstracts; 12 poster presentations; 1 popular press interviews; 3 podcasts; 1 grower meeting presentations; and 3 seed dealer presentations;
	Stripe rust resistant lines	In 2020, all lines under field testing were screened for six stripe rust resistance markers to identify presence of genes useful in the PNW. New populations segregating for resistance to Yr5 and Yr15 were screened and selected for advancement.	Each year new crosses are made to stripe rust resistant lines. These are subsequently developed, screened, and advanced to state-wide yield trials. At any given time, lines are in every stage of development	
	End-use quality lines	In 2020, populations that were selected for combinations of the GBSS genes (waxy) and the glutenin genes were advanced to field testing. All breeding lines are screened for the presence of low PPO genes, and populations were advanced in the greenhouse of lines containing none of the PPO genes.	Each year new crosses are made to lines containing unique end-use quality genes. These are subsequently developed, screened, and advanced to state-wide yield trials. At any given time, lines are in every stage of development	
	Reduced height lines	In 2020, all breeding lines in field trials were screened to identify which dwarfing gene they carry in order to aid in selection and crossing decisions. Selection is then made on which genes are present rather than incorporating new genes as they already exist in our breeding program. All lines are field tested for emergence potential.	Each year, we verify presence of dwarfing genes in all material to assist with selection of lines with enhanced emergence potential.	

	Genomic selection	With the assistance of Dr. Lozada (post-doctoral researcher) and graduate students, we have continue to build genomic prediction models for traits of interest. Lines from the 2015-2020 breeding program have been genotyped and used for model building. We have begun incorporating high-throughput phenotyping measurements in these selection models, which has improved selection accuracy and efficiency.	Each year we will continue to phenotype the training panel, add more lines to the training panel (and genotype them), and refine the prediction model. Validation of results is proceeding.	Results are presented through annual progress reports, the research review, field tours, and grower meetings
Genotyping advanced breeding lines	Provide useful information regarding genetic diversity and gene profiles to better estimate crossing potential	In 2020, the advanced germplasm was screened with DNA markers for about 25 markers of interest. This information was used to enhance selection of field tested material, as well as assist in parent cross-combinations to develop populations with desired traits of interest.	This is done annually	Results are presented through annual progress reports, with the outcomes of this research being realized in new cultivars
Greenhouse				Results are presented through annual progress reports, with the outcomes of this research being realized in new cultivars
	Hybridization and propagation	In 2020 we made approximately 650 crosses which were targeted for herbicide resistance, low rainfall and high rainfall production. About 80% of these are in soft white backgrounds, and the remaining in hard red backgrounds. Crosses were advanced to the F2 stage. We also made about 100 crosses for trait introgression to continue to build germplasm for traits which are important to PNW growers.	This is done annually, with the number of crosses/populations varying	
	Single-seed descent	In 2020 we began developing more SSD populations to better standardize the production of lines from our crossing program. We are fine-tuning the protocols to maximize the number of lines which can be tested, and are looking forward to seeing how this new process assists the breeding program.	This is done annually, with the number of crosses/populations varying	
	Doubled haploid	In 2020 our DH production focused on lines which were not completed in 2019. We continued to advance lines which were produced in 2019 to generate more seed before they went into field testing. The goal is to have all lines produce go into 4-row observation trials at both Pullman and Lind.	This is done annually, with the number of crosses/populations varying	

	Trait Introgression	We made crosses to germplasm containing resistance/tolerance to snow mold, stripe rust, end use quality, foot rot resistance, preharvest sprouting, Al tolerance, Ceph Stripe, SBWMV, vernalization duration, low PPO, Fusarium head blight, imazamox, the CoAxiom system, and other herbicides (in coordination with Dr. Burke). Herbicide tolerant lines are screened in the greenhouse for tolerance, as well as with markers, before going into field trials. We now have markers for many of these traits, and can efficiently screen for their presence. After advancement, all populations are transferred to the field program to undergo further testing.	This is done annually, with the number of crosses/populations varying	
Trait assessment				Results are presented through annual progress reports, with the outcomes of this research being realized in new cultivars
	Coleoptile length	Lines are screened and selected for coleoptile length.	Screening and selection will continue in 2021. Superior lines were planted in the field and crossed back into the breeding program.	
	Herbicide Tolerance	We now have a strong pipeline of germplasm tolerant to imazamox, and continue to develop and screen populations. We have many soft white lines using the CoAxiom system under field evaluation, and continue to make crosses for this trait. We have expanded to select for both hard and soft germplasm, and work with all three tolerance genes. In collaboration with Dr. Burke, we have new sources of herbicide tolerance which are being tested under both greenhouse and field conditions for tolerance.	Screening and selection will continue in 2021. Superior lines were planted in the field and crossed back into the breeding program.	
	Cold Tolerance	All advanced breeding lines are screened for cold tolerance through the USDA funded WGC grant.	Screening and selection will continue in 2021. Superior lines were planted in the field and crossed back into the breeding program.	

	Stripe rust	Previously, we identified over 20 QTL in PNW germplasm, about half of which appear to be novel. These lines are now being crossed to additional breeding lines and cultivars, and selection will be done with the recently identified markers to incorporate this resistance through a diversity of backgrounds. Because many sources of resistance in our germplasm are uncharacterized, we have begun developing genomic selection models to use for selection in our breeding populations. We have started the development of more populations to identify more of the genes which are contributing to resistance in our germplasm.	Screening and selection will continue in 2021. Superior lines were planted in the field and crossed back into the breeding program.	
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**Washington Grain Commission
Wheat and Barley Research Annual Progress Reports and Final Reports**

Project #: 6195

Progress Report Year: 3 of 3

Title: Field Breeding Soft White Winter Wheat

Investigator/Cooperators: AH Carter, KG Campbell, XM Chen, CF Morris, TD Murray

Executive summary: Two new cultivars, Piranha CL+ and Sockeye CL+, were approved for release in September, 2020. Both lines have improved disease resistance (snow mold tolerance, stripe rust resistance, and strawbreaker foot rot tolerance) and yield potential over the two most widely grown Clearfield cultivars in production. Piranha CL+ is a widely adapted line and performs best under deep-furrow plantings. Sockeye CL+ is also widely adapted, and has maximum yield potential under high input systems. Both lines had strong demand for foundation seed, and are being increased by seed dealers as rapidly as possible. Recent releases of Stingray CL+ and Pritchett were planted in 2020 for commercial production, and were in high demand. Castella and Devote are in their final stages of seed increase and will be available for commercial production in 2021. These recent releases all have high grain yield, disease resistance, and good end-use quality. Production of these lines will mitigate concerns growers have with current cultivars. Many new lines have been entered into Variety Testing trials in 2021, and are also on breeder seed increase. Promising lines are also being advanced with the CoAXium system, and are in statewide trials. Over 2,000 unreplicated yield-trial plots were evaluated at either Pullman or Lind and thousands of F4 head rows and DH rows were evaluated in Pullman, Lind, and Waterville. Over 1,500 DH lines were planted for 2020 evaluation. High selection pressure is continually placed on disease resistance, emergence, flowering date, end-use quality, straw strength, etc. Multiple screening locations have been established to evaluate germplasm for: stripe rust resistance, foot rot resistance, snow mold resistance, good emergence, aluminum tolerance, soil borne wheat mosaic virus resistance, Cephalosporium tolerance, and nematode resistance. Some of these locations were not planted in the fall of 2020 due to reductions in the FY2021 budget request. We continue to put a strong emphasis on soft white wheat in the program, and have modified our breeding schemes to account for marker-assisted selection, genomic selection, and doubled-haploid production. We have increased the number of locations we test at to account for new herbicide resistant lines (non-imazamox) and to better evaluate lines across production regions in Washington.

Impact: Traditionally, over 85% of the wheat crop in our state is soft white wheat. Even very small reductions of required grower input and/or increases in productivity can mean millions of dollars to the growers, grain trade and allied industries. By providing genetic resistance to diseases and increasing agronomic adaptability, input costs will be reduced and grain yield increased. WSU soft white cultivars are grown on approximately 40% of the acres. These include Bruehl, Eltan, Xerpha, Otto, Puma, Jasper, Purl, Curiosity CL+, Mela CL+, and Resilience CL+. Measured impact is demonstrated with performance of past cultivars, upcoming production of recent cultivars (Pritchett, ARS-Castella, Stingray CL+, Devote) and strong interest in new cultivars (Piranha CL+ and Sockeye CL+).

WGC project number: 6195
WGC project title: Field Breeding Soft White Winter Wheat
Project PI(s): AH Carter
Project initiation date: July 1, 2009
Project year: 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
Develop soft white winter wheat cultivars	New cultivars released for production in WA	We released the soft white lines Otto, Jasper, Puma, Purl, Stingray CL+, Devote, Piranha CL+, and Sockeye CL+. Collaborative releases include Curiosity CL+, Mela CL+, Resilience CL+, Pritchett, and ARS-Castella. All lines are being commercially produced or are in seed increase for commercial production. We have multiple breeding lines in statewide testing for consideration of release, many of which had excellent performance in 2020. We have over 16,000 plots and 30,000 rows of soft white material under evaluation at various stages of the breeding process.	Each year we evaluate germplasm at each stage of the breeding process. Each year lines are entered into statewide testing for final release consideration. A cultivar is released, on average, every two years.	Progress will be reported through field days, grower meetings, commission reports, annual progress reports, and peer-reviewed manuscripts
	Agronomic traits	We have 18 locations across the state representing diverse climatic zones in which advanced breeding lines are evaluated for agronomic characteristics. Early generation material is selected for in Lind and Pullman. This year we moved all DH production to initial 4-row selections due to the ability to screen for important traits such as emergence and stripe rust, along with our snow mold screening in Waterville.	Evaluation is done annually at multiple locations across the state.	In 2020 we communicated results of this project through the following venues: 14 peer-reviewed publications; 3 virtual field day recordings; 6 field day abstracts; 12 poster presentations; 1 popular press interviews; 3 podcasts; 1 grower meeting presentations; and 3 seed dealer presentations;
	Disease resistance	Disease resistance is recorded on our 18 breeding locations as disease is present, with certain locations being selected specifically for disease pressure (Waterville for snow mold, Pullman for stripe rust, etc.). Additional locations are planted in cooperation with plant pathologists to screen other diseases of importance in WA.	Evaluation is done annually at multiple locations across the state.	

	End-use quality	All F4/DH and greater material is subjected to end-use quality screens to evaluate performance. Lines with poor quality are discarded from the breeding program and from selection in 2020.	Each year, all head rows are evaluated for end-use quality and lines predicted to have superior quality advanced. Each yield trial is submitted for quality evaluations and those with high performance are advanced in the breeding process.	
	Herbicide resistance	Multiple soft white lines have been developed for herbicide resistance and are being evaluated under replicated trials across the state. We have multiple Clearfield lines, advanced lines in testing for the CoAXium system, and novel traits are being incorporated into germplasm and field tested through collaboration with Dr. Ian Burke.	Evaluation is done annually at multiple locations across the state.	
Introgress novel genes for essential traits	Incorporation of novel genes into adapted germplasm for evaluation under WA environments			Progress will be reported through field days, grower meetings, commission reports, annual progress reports, and peer-reviewed manuscripts
	Rht and photoperiod genes	Crosses have been made to include non-traditional Rht and photoperiod genes into our soft white winter wheat germplasm for testing under PNW conditions.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Stripe rust genes	We constantly have material coming out of the MAS program for stripe rust. In 2020 we evaluated multiple populations in both early and preliminary yield trials. Material includes new genes identified from Eltan, Coda, and novel genes from GWAS analysis.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Foot rot genes	We have many populations being screened for foot rot resistance. Field evaluations of these selections are done in collaboration with Dr. Campbell.	Crosses made through the project #5195 will be evaluated under field conditions upon MAS.	
	Cephalosporium	No markers are currently being used for this introgression. All selection is being done under field conditions. We recently made many crosses to resistant material and are now field screening them for selection of resistant material.	Evaluation were done in field locations in WA in 2020	
	Aluminum tolerance	Field screening of breeding lines for aluminum tolerance is being conducted under field conditions. We recently made many crosses with material that was aluminum tolerant. Screening of this material will be completed in 2020.	Evaluation were done in field locations in WA in 2020	

	Hessian Fly	In collaboration with Dr. Nilsa Bosque-Perez and Arash Rashed we screened 12 F2 populations with new sources of resistance to Hessian Fly. Resistant plants were returned to the breeding program for further crossing and segregating populations are currently being screened again for resistance. Selected lines were planted for field evaluations in 2021.	Additional populations will be developed in 2021. Current populations will be field evaluated in 2021.	
	Nematodes	Nematode screening has been done in collaboration with Dr. Paulitz and Dr. Campbell. Additional advanced material will be screened in 2020.	Populations were screened in 2020	
	End-use quality	Lines are continually screened for end-use quality. We submitted an additional 10 lines for statewide testing to begin generating quality scores prior to release decisions.	Validated genomic prediction models were available for selection in 2020.	

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**Washington Grain Commission
Wheat and Barley Research Annual Progress Report**

Project #: 3144

Progress Report Year: 2 of 3 (2020)

Title: Improving Control of Rusts of Wheat and Barley

Cooperators: K. Garland Campbell, A. Carter, M. Pumphrey, & D. See

Executive summary: During 2020, studies were conducted according to the objectives of the project proposal, and all objectives specified for the second year have been successfully completed. In addition to the major accomplishments and their impacts listed below, this project results in genetic resources and techniques for further studying the biology and genetics of the pathogens, stripe rust resistance, and mechanisms of interactions between the pathogens and plants.

Impact: 1) Stripe rust was accurately forecasted and monitored through the 2020 crop season, and rust updates and advises were provided on time to growers, which successfully prevented a severe epidemic potentially causing yield losses of 11% on both winter and spring wheat varieties on average in Washington state. 2) We identified 19 (including 2 new) races of the wheat stripe rust pathogen and 8 (including 1 new) races of the barley stripe rust pathogen from the 2020 collection in Washington. The virulence information is useful in developing and screening resistant varieties. 3) We characterized the US stripe rust collections from 2010 to 2017 using 14 SSR markers. From 2,414 isolates, we identified 1,599 multi-locus genotypes (MLGs) and studied the genetic diversity and population differentiation. The results improve the understanding of stripe rust epidemiology and spore movement among different regions in the US. 4) We completed a study of whole genome sequencing for a sexual recombinant population of the wheat stripe rust pathogen and identified candidate genes for avirulence. Using the gene sequences, we are developing virulence-specific markers for monitoring race changes in the pathogen population. 5) We evaluated more than 25,000 wheat, barley, and triticale entries for resistance to stripe rust. From the tests, we identified new sources of resistance and resistant breeding lines for breeding programs to release new varieties for growers to grow. In 2020, we collaborated with breeders in releasing, pre-releasing, or registered 19 wheat and 1 barley varieties. The germplasm evaluation data were also used to update the Seed-Buying Guide for growers to choose resistant varieties to grow. 6) We completed genome-wide association studies and mapped 37 genes in the spring wheat panel and 51 genes in the winter panel for stripe rust resistance. These results provide information on which genes are effective and used in breeding programs in various regions in the US, especially the Pacific Northwest. The resistance genes and their markers are useful in breeding stripe rust resistant wheat varieties. 7) We tested 19 fungicide treatments for control of stripe rust on winter and spring wheat and provided the data to chemical companies for registering new fungicides and tested 23 winter wheat and 23 spring wheat varieties for yield loss caused by stripe rust and yield increase by fungicide application. The data of the fungicides and varieties are used for guiding the integrated control of stripe rust. 8) In 2020, we published 30 journal articles and 6 meeting abstracts.

Outputs and Outcomes:

Project PI(s): Xianming Chen				
Project initiation date: 7/1/2019				
Project year: 2 of 3 (2020)				
Objective	Deliverable	Progress	Timeline	Communication
1. Improve the understanding of rust disease epidemiology and the pathogen populations.	1) New races. 2) Information on distribution, frequency, and changes of all races, and possible fungicide tolerant strains. 3) New tools such as molecular markers and population structures. The information will be used by breeding programs to choose effective resistance genes for developing new varieties with adequate and durable resistance. We will use the information to select a set of races for screening wheat and barley germplasm and breeding lines. The information is also used for disease management based on races in different regions.	All planned studies for the project in 2020 have been completed on time. There is not any delay, failure, or problem in studies to this objective. The race identification for the 2019 collection was completed and summarized. The data and summary were sent to growers, collaborators and related scientists in May 2020. In the 2020 crop season, we collected and received 350 stripe rust samples throughout the country, of which 217 samples (65%) were collected by ourselves from Washington. We have completed the race ID work for all samples of the 2020 collection and detected 19 wheat stripe rust races (including 2 new races) and 11 barley stripe rust races (including 2 new races), of which all 19 wheat and 8 (including 1 new race) barley stripe rust races were detected in Washington. The distribution and frequency of each race and virulence factor in WA and the whole country have been determined. Predominant races have been identified. The information on races and virulence factors is used to guide breeding programs for using effective resistance genes in developing resistant varieties and selected predominant races with different virulence patterns are used in screening breeding lines for stripe rust resistance. We have characterized the US stripe rust collections from 2010 to 2017 using 14 SSR markers. From 2,414 isolates, we identified 1,599 multi-locus genotypes (MLGs) and studied the genetic diversity and population differentiation. Using the same set of markers, we have been genotyping the 2018 - 2020 collections. We completed a study of whole genome sequencing for a sexual recombinant population of the wheat stripe rust pathogen and identified candidate genes for avirulence. Using the gene sequences, we are developing virulence-specific markers for monitoring race changes in the pathogen population. These studies have improved the understanding the epidemiology, biology and genetics of the pathogen, and provided information and resources for improvement of monitoring and managing stripe rusts on wheat and barley.	The race identification work for the 2019 stripe rust samples was completed and summarized. The race identification work for the 2020 samples was completed, and analyses and summarization are underway. Molecular work for the population genetic studies has been completed up to the 2017 collection, and almost completed for the 2018 collection. DNA extraction of the 2019 samples has been completed and of the 2020 samples are underway.	The rust race data were communicated to growers and researchers through e-mails, websites, project reports, meeting presentations and publications in scientific journals (for detailed information, see the lists in the main report file).

<p>2. Improve rust resistance in wheat and barley varieties.</p>	<p>1) Stripe rust reaction data of wheat and barley germplasm and breeding lines. 2) Reactions to other diseases when occur. 3) New resistance genes with their genetic information and molecular markers. 4) New germplasm with improved traits. 5) New varieties for production. The genetic resources and techniques will be used by breeding programs for developing varieties with diverse genes for stripe rust resistance, which will make the stripe rust control more effective, efficient, and sustainable.</p>	<p>In 2020, we evaluated more than 25,000 wheat, barley, and triticale entries for resistance to stripe rust. The entries included germplasm, breeding lines, rust monitoring nurseries, and genetic populations from various breeding and extension programs. All nurseries were planted and evaluated at both Pullman and Mt. Vernon locations under natural stripe rust infection. Some of the nurseries were also tested in Walla Walla and Lind, WA. Germplasm and breeding lines in the variety trial and regional nurseries also were tested in the greenhouse with selected races of stripe rust for further characterization of resistance. Disease data of regional nurseries were provided to all breeding and extension programs, while data of individual breeders' nurseries were provided to the individual breeders. Through these tests, susceptible breeding lines can be eliminated, which should prevent risk of releasing susceptible cultivars and assisted breeding programs to release new cultivars of high yield and quality, good adaptation, and effective disease resistance. In 2020, we collaborated with public breeding programs in releasing and registered 19 wheat varieties and 1 barley variety. Varieties developed by private breeding programs were also resulted from our germplasm screening program. Through our evaluation, we have established a collection of wheat germplasm with stripe rust resistance, which are valuable sources of stripe rust resistance for further characterization of resistance, identified new effective resistance genes, and for development of wheat varieties with effective resistance. Through our intensive testing, varieties with durable resistance to stripe rust have been developed. In 2020, we completed several studies for mapping stripe rust resistance genes. We mapped two major genes on chromosomes 1AL and 3BL and one minor gene on 2AL in wheat landrace PI 197734 using a doubled haploid population and developed user-friendly KASP markers for the two major genes to be used in breeding programs. We completed two whole-genome associate studies (GWAS) and mapped 37 loci in a panel of 616 spring wheat cultivars and breeding lines and identified 51 loci in a panel of 857 winter wheat cultivars and breeding lines developed in the US. We determined the frequencies and distributions of stripe rust resistance genes used in various regions in the US with the PNW had the highest number of genes. In a study involving 40 crosses of winter wheat varieties, we obtained preliminary results of identifying more than 220 SNP markers at least 73 loci for stripe rust resistance using the bulking segregation analysis and genotyping by multiplex sequencing. In 2020, we phenotyped 10 of the crosses for stripe rust reaction using the F6 generation to validate resistance loci. We selected new wheat germplasm lines with single new genes or combinations of genes for resistance to stripe rust to make them available for breeding programs and directly provided seeds to some US breeding programs.</p>	<p>All 2020 germplasm tests were completed, and the data were provided to collaborators on time. The 2020-21 winter wheat nurseries were planted in fields in September and October 2020. The 2020 spring crop nurseries will be planted in March-April 2020. The greenhouse tests of the 2020 spring nurseries and the 2020-21 winter wheat nurseries have been conducted in the greenhouse during the winter, and will be completed by May 2020. All experiments of the molecular mapping studies scheduled for 2020 have been completed. Mapping populations of winter wheat were planted in fields in October 2020 and those of spring wheat will be planted in April, 2021 for stripe rust phenotype data. Populations with adequate phenotype data are genotyped with molecular markers for mapping resistance genes.</p>	<p>The data of variety trials and regional nurseries were sent to growers and collaborators through e-mails and websites. Summary information of varieties were sent to growers and collaborators through rust updates and recommendations through e-mails, websites, Seed-Buying Guide, variety release documents. Test data of individual breeding programs were sent to the individual breeders. New genes and molecular markers were reported in scientific meetings and published in scientific journals (see the publication and presentation lists in the report main file).</p>
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<p>3. Improve the integrated management of rust diseases.</p>	<p>1) Data of fungicide efficacy, dosage, and timing of application for control stripe rust. 2) Potential new fungicides. 3) Stripe rust yield loss and fungicide increase data for major commercial varieties. 4) Stripe rust forecasts and updates. 5) Guidance for rust management. The information is used for developing more effective integrated control program based on individual varieties. Disease updates and recommendations will allow growers to implement appropriate control.</p>	<p>In 2020, we evaluated 19 fungicide treatments on winter wheat and spring wheat, plus non-treated checks, for control of stripe rust in experimental fields near Pullman, WA. On winter wheat, all fungicide treatments significantly reduced stripe rust severity compared to the non-treated check, and six of them provided the best control of stripe rust. All treatments had higher test weight than the non-treated check, and the treatments of two applications had higher test weight than those of one application. All treatments produced grain yields higher than the non-treated check with nine treatments producing the highest yields. The significant yield responses of the fungicide treatments ranged from 21.8 to 59.7 bushels per acre or 42.1 to 115.3% more grain. On spring wheat, all fungicide treatments significantly reduced overall stripe rust severity compared to the non-treated check. All 15 treatments with applications at both middle jointing stage and heading stage provided equally better control of stripe rust than the 4 treatments with only one application at the heading stage. All treatments had higher test weight than the non-treated check. All treatments produced grain yields higher than the non-treated check, and treatments with two applications generally produced higher yields than those with only one application at the heading stage. The significant yield responses ranged from 34.4 to 51.0 bushels per acre or 68.4 to 101.4% more grain. These results are useful for managing stripe rust on both winter and spring wheat crops. In 2020, we tested 23 winter wheat and 23 spring wheat varieties commonly grown in the PNW, plus highly susceptible checks. For winter wheat, stripe rust caused yield loss of 62.4 bu/A (48.6%) on the susceptible check and from 1.2 to 52.4 bu/A or 0.7 to 35.7% (average 11.6%) on commercially grown varieties. Fungicide application increased yield by 0.7 to 55.4% (average of 14.3%) on commercially grown winter wheat varieties. For spring wheat, stripe rust caused 83.8% yield loss on the susceptible check and from 0 to 62.8 bu/A or 0 to 57.7% (average 10.7%) yield losses on commercial varieties. Fungicide application increased grain yields by 0 to 136.5% (average 13.1%) on commercial varieties. These results can be used by chemical companies to register new fungicides and used by growers for selecting resistant varieties to grow and use suitable fungicide application for control stripe rust on varieties without an adequate level of resistance. Accurate forecast of wheat stripe rust epidemic was made based on prediction models, rust monitoring was conducted, and recommendations were made through the 2020 crop season, which successfully prevented major yield losses and saved growers millions of dollars.</p>	<p>For this objective, all tests scheduled for 2020 were successfully completed. For the 2020-21 growing season, the winter wheat plots of the fungicide and variety yield loss studies were planted in October 2020, and the spring plots will be planted in April 2021. The tests will be completed in August (for winter wheat) and September (for spring wheat), 2021.</p>	<p>The results were communicated to growers and collaborators through e-mails, presentations in growers meetings, project reports and reviews, and published in scientific journals (see the publication and presentation lists in the report main file).</p>
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Publications:

Scientific Journals:

Liu, Y., Qie, Y. M., Li, X., Wang, M. N., and **Chen X. M.** 2020. Genome-wide mapping of quantitative trait loci conferring all-stage and high-temperature adult-plant resistance to stripe rust in spring wheat landrace PI 181410. *International Journal of Molecular Sciences* 21:478(1-18). <https://doi.org/10.3390/ijms21020478>

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Popular Press Articles:

January 7, 2020. 2020 First Stripe Rust Forecast and 2019 Fungicide and Variety Yield Loss Tests. Xianming Chen. E-mail sent to cereal group. <https://striperust.wsu.edu/2020/01/21/2020-first-stripe-rust-forecast-and-2019-fungicide-and-variety-yield-loss-tests-january-7-2020/>

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October 8, 2020. 2020 Variety Yield Loss Tests. Xianming Chen, Kent Evans, and Jason Sprott. E-mail sent to cereal group. <https://striperust.wsu.edu/2020/10/08/2020-variety-yield-loss-tests/>

Presentations and Reports:

In 2020, Xianming Chen presented invited talks at the following national and international meetings:

“Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2019.” Poster presentation (authored by Wang, M. N. and Chen, X. M.) at the 2020 American Phytopathological Society Annual Meeting, August 9-12, 2020 (Virtual meeting)

“Stripe rust resistance genes in U.S. wheat cultivars and breeding lines identified by molecular markers and genome-wide association studies.” Poster presentation (authored by Liu, L., Mu, J. M., Liu, Y., Wang, M. N., See, D. R., and Chen, X. M.) at the 2020 American Phytopathological Society Annual Meeting, August 9-12, 2020 (Virtual meeting)

“Population differentiation, diversity, and dynamics of stripe rust pathogen *Puccinia striiformis* on wheat and barley in the United State from 2010 to 2017.” Student oral presentation (authored by Bai, Q., Wan, A. M., Wang, M. N., See, D. R., and Chen, X. M.) at the 2020 American Phytopathological Society Annual Meeting, August 9-12, 2020 (Virtual meeting)

“Potential risks of *Puccinia striiformis* and *P. graminis* infection on barberry in Asia and Southeastern Europe.” Oral presentation (authored by Chen, X. M., and Sinha, P.) at Borlaug Global Rust Initiative Technical Workshop 2020. October 8, 2020 (Zoom meeting).

<https://bgri.cornell.edu/2020-bgri-technical-workshop-additional-presentations/>

“Identification of a large number of stripe rust resistance loci in U.S. spring and winter wheat cultivars and breeding lines using genome-wide association mapping and *Yr* gene markers.” Poster presentation (authored by Chen, X. M., Liu, L., Mu, J. M., Wang, M. N., See, D. R.) at Borlaug Global Rust Initiative Technical Workshop 2020. October 7-9, 2020 (Zoom meeting). <https://bgri.cornell.edu/wp-content/uploads/2020/10/poster-chen.pdf>

“Genomic selection for ordinal stripe rust resistance traits. Abstracts of 2020 Borlaug Global Rust Initiative Technical Workshop.” Poster presentation (authored by Merrick, L. F., Chen, X. M., Ward, B. P., and Carter, A. H. at Borlaug Global Rust Initiative Technical Workshop 2020. October 7-9, 2020 (Zoom meeting). <https://bgri.cornell.edu/wp-content/uploads/2020/10/poster-merrick.pdf>

Reports:

Report of race summaries of the 2019 stripe rust collection in the US. May 2020, <https://striperust.wsu.edu/races/data/>

Stripe data of more than 50 wheat and barley germplasm nurseries tested in 2020. <https://striperust.wsu.edu/nursery-data/2020-nursery-data/>

Chen, X. M., Evans, K. C, Wang, M. N., Sprott, J., Liu, Y., Liu, L., Li, Y. X., and Mu, J. M. 2020. Cereal rust management and research in 2019. Page 50 in: 2020 Field Day Abstracts: Highlights of Research Progress, <http://s3-us-west-2.amazonaws.com/css.wsu.edu/wp-content/uploads/2020/06/15144922/FDA-2020.pdf>

Merrick, L. F., Carter, A. H., Chen, X. M., Ward, B. P. 2020. Genomic selection of stripe rust resistance in a wheat breeding program. Page 64 in: 2020 Field Day Abstracts: Highlights of Research Progress, <http://s3-us-west-2.amazonaws.com/css.wsu.edu/wp-content/uploads/2020/06/15144922/FDA-2020.pdf>

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

PROJECT #: 30109-3156

Progress report year: 2 of 3

Title: Club wheat Breeding

Researcher(s): Kimberly Garland Campbell, Arron Carter

Cooperator: Mike Pumphrey

Executive summary:

Pritchett was grown on 15,183 acres in 2020. Castella club wheat was available as registered seed in fall of 2020. New entries with better resistance to snow-mold, including one IMI-club, were entered in the WA variety trials in the dry zone. New entries with earlier maturity, excellent standability, excellent club wheat quality and resistance to stripe rust were entered into the WA variety trials in the high rainfall zone. With our collaborators, we evaluated nurseries at 12 locations in Washington, Idaho and Oregon. This year we evaluated all of plots in our yield trials in Lind and Pullman for resistance to low falling number using spike wetting tests. This data was available prior to planting so that we could make selections. Pritchett was rated as resistant to soil borne mosaic virus over multiple years of testing by Dr. C. Hagerty of OSU. We evaluated over 2500 breeding lines from around the U.S. for resistance to stripe rust. We made crosses to several good sources of Hessian fly and wheat virus resistance and these are being advanced for selection. We evaluated mini-bulk breeding and speed breeding techniques in the greenhouse and discovered that we can save about 20 days off of normal winter wheat generations using these techniques which allows us to advance material through the greenhouse faster and serves as a cost effective alternative to doubled haploids. We were unable to visit with our collaborators of the Japanese Flour Miller's Association in person in 2020 due to the pandemic but we were able to meet in a virtual conference in July 2020. From that interaction, we are investigating additional methods to measure cake quality including image analysis and texture classification. We entered the following lines into the WA variety testing trials in 2021: ARS09X492-6CBW, ARS14DH1122-26, ARS09500-17CBW, ARSX12015-68CBW, ARSX190997-8D, WA8317. We currently plan to propose ARS09X492-6CBW for release targeted to the Palouse. WA8317 is a C12 line developed in conjunction with the WSU Winter Wheat program and will also likely be proposed for release.

Impact

The integration of genomic selection, speed breeding, doubled haploid breeding, and new methods of analyzing data enables us to continue to be efficient with grower dollars and produce club wheat cultivars that are competitive as well as additional soft wheat germplasm with specific useful traits for other breeders. Better resistance to low falling number will stabilize markets and reduce grower risk. Growers will maximize choice in marketing strategy.

**Summaries from 2020 WSU Cereal Variety Testing Program
By Rainfall Zone. Club Wheats are in Italics**

<i>Name</i>	>20 in.					16-20 in.				
	<i>Colton</i>	<i>Fairfield</i>	<i>Farmington</i>	<i>Pullman</i>	<i>Ave.</i>	<i>Dayton</i>	<i>Mayview</i>	<i>St John</i>	<i>Walla Walla</i>	<i>Ave.</i>
<i>ARS Crescent</i>	130	135	137	124	131	127	104	130	132	123
<i>ARS09X492-6CBW</i>	125	137	139	144	136	124	98	128	132	121
<i>ARSX12016-45CBW</i>	129	125	132	147	133	120	97	115	124	114
<i>Castella</i>	125	130	131	145	133	123	57*	115	134	107
<i>OR5170022</i>	107	114	114	118	113	115	92	117	118	110
<i>Pritchett</i>	125	130	131	145	133	131	97	124	132	121
M-press	140	142	131	142	139	131	110	119	146	127
Norwest Duet	125	130	131	145	133	126	111	127	138	126
Purl	125	130	131	145	133	132	94	130	139	124
Isd	16	11	17	15	6	13	15	11	21	7

<i>Name</i>	12-16 in.						<12 in.					
	<i>Almira</i>	<i>Anatone</i>	<i>Creston</i>	<i>Lamont</i>	<i>Reardan</i>	<i>Ave.</i>	<i>Bickleton</i>	<i>Connell</i>	<i>Harrington</i>	<i>Horse</i>	<i>Heaven Lind</i>	<i>Ave</i>
<i>ARS Crescent</i>	77	75	73	95	127	89	27	69	67	40	70	56
<i>ARS12015-68CBW</i>	64	75	75	85	111	82	24	61	57	36	60	48
<i>Bruehl</i>	73	66	81	88	101	82	25	64	54	39	58	47
<i>Castella</i>	74	59	88	95	119	87	25	56	51	38	60	46
<i>OR5170022</i>	64	70	79	84	97	79	19	60	41	29	60	42
<i>Pritchett</i>	81	73	72	95	117	88	29	62	54	39	59	49
<i>WA8317</i>	69	69	84	85	106	83	26	61	60	38	58	49
Otto	66	72	81	94	108	84	27	57	49	39	57	46
Devote	66	70	88	89	108	85	25	64	60	45	61	51
M-press	79	71	95	91	122	92	29	68	57	38	56	50
Norwest Duet	82	78	78	95	122	91	32	67	68	37	59	53
Purl	74	83	82	88	114	88	27	65	51	40	60	48
Isd	10	11	13	8	14	4	3	14	15	8	12	4

* The low score at Mayview for Castella is likely due to deer and elk feeding.

** Shaded entries are in the same highest Isd group.

WGC project number: 3019-3156
WGC project title: Club wheat breeding
Project PI(s): Kimberly Garland-Campbell and Arron Carter
Project initiation date: 7/1/19
Project year: 2 of 3

Objective	Deliverable	W	Timeline	Communication
1. Develop agronomically competitive club wheat cultivars targeted to the diversity of rainfall and production zones of the PNW. These cultivars will possess the excellent end use quality characteristic of club wheat. They will also possess excellent resistance to stripe rust. Specific other characteristics will be targeted to individual rainfall regions (see below).	Club cultivar releases	Four club wheat breeding lines were entered into the high rainfall Washington Cereal Variety Testing nurseries. IN the high rainfall zone, ARS09X492-6CBW and ARSX12016-45CBW were entered into the high rainfall zone and ARS12015-68CBW and WA 8317 were entered into the low rainfall nurseries. ARS09X492-6CBW, adapted to the Palouse, and WA8317, a 2 gene IMI club wheat that is adapted to the dry region, will be proposed for release. The following lines were entered into WA variety testing trials in 2021: ARS09X492-6CBW, ARS14DH1122-26, ARS09400-17CBW, ARSX12015-68CBW, ARSX190997-8D, WA8317.	June 2019-June 2022. Cutlvar releases are targeted as one every three years per rainfall zone.	Presentation at grower meetings, Wheat commission meetings, field days, plot tours, Wheat Life and Research Review. Plant registrations manuscripts. Virtual field days in conjunction with Dr. Clark Neely.
A. Develop club breeding lines and cultivars for the <15 inch rainfall zone with improved resistance to snow mold and fusarium crown rot, improved emergence and winter survival.	Club cultivar releases and breeding lines entered into Western Regional and state extension trials.	We evaluated snow mold resistance at Waterville an Mansfield in collaboration with the WSU winter wheat breeding program. The club breeding line ARSX12015-68CBW, has above average resistance to snow mold and is being tarted to the area north of US Highway 2 in Washington. Results for fusarium evaluation will be reported in that project.	Sept 2016-June 2019.	Presentation at grower meetings, Wheat commission meetings, field days, plot tours, Wheat Life and Research Review. Peer reviewed paper on molecular approaches to breeding for soil born disease.
B. Develop club breeding lines and cultivars for the >15 inch rainfall zone with improved resistance to eyespot, cephalosporium stripe, aluminum toxicity, and cereal cyst nematodes.	Club cultivar releases and breeding lines entered into Western Regional and state extension trials.	We initiated a collaboration with Univ. of Idaho to evaluate populations that are segregating for Al tolerance at the U.I.Parker Farm in a location with high levels of exchangeable Al. Results will be available this spring and summer.	Sept 2016-June 2019.	Presentation at grower meetings, Wheat commission meetings, field days, plot tours, Wheat Life and Research Review.
C. Release a club breeding lines and cultivars with early spring green up, targeted to SE Washington.	Club cultivar releases and breeding lines entered into Western Regional and state extension trials.	Each year we plant headrows and plots at Pendleton and Walla walla and rated growth prior to and just at heading to target earlier heading cultivars. Club wheat sister lines, ARS090500-14CBW and ARS09500-17CBW, performed well early environments and were entered into the Oregon and Washington state extension trials for 2021.	Sept 2016-June 2019.	Presentation at grower meetings, Wheat commission meetings, field days, plot tours, Wheat Life and Research Review.
Objective 2. Release germplasm and cultivars with the excellent end use quality characteristic of club wheat and with resistance to preharvest sprouting and late maturity alpha amylase (LMA)	Club wheat breeding lines with stable falling numbers above 300 in all but extreme environments.	We applied factor analysis to the data collected by Camille Steber and ranked cutlivars and breeding lines for resistance to falling number. In conjunction we harvested spikes at physiological maturity from 2020 field plots and worked with the Steber lab group to rate early and mid level breeding plots for resistance to preharvest sprouting. This provided data that we could use to make selections prior to planting.	Sept 2016-June 2019.	Presentation at grower meetings, Wheat commission meetings, field days, plot tours, Wheat Life and Research Review. Direct communication with breeders. Peer reviewed papers.

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

PROJECT #: 30109-3157

Progress report year: 2 of 3

Title: Evaluation and Selection for Cold Tolerance in Wheat

Cooperators: K. Garland Campbell, K. Sanguinet, A.H. Carter

Executive summary:

In 2020, in spite of the slow down due to the pandemic, we rated 924 breeding lines and cultivars from public winter wheat breeding and variety testing programs for survival. This compared well with 2019 when we tested 936 lines. Breeders used this information for selection of new experimental lines. We have evaluated the Washington Extension Winter Wheat Trials every year since 2001. The survival results for the top varieties grown according to the Washington Grain Commission Variety Survey are below (next page). We observed association between our testing results and the winter injury that occurred in WA in April of 2020.

We conducted a genome-wide association study of wheat from the WSU winter wheat and ARS winter wheat programs and a doubled haploid population of Cara/Xerpha. We also did a preliminary QTL analysis on a population derived from the very cold tolerant Canadian cultivar, Norstar, in collaboration with Dr. Debbie Laudencia of USDA-ARS in Albany CA. We previously identified allelic variation at the Vrn and CBF genes on the group 5 chromosomes that impacts vernalization requirement and cold tolerance. We have now identified QTL for cold tolerance on chromosomes 1A, 2A, 4A, 5B, 7A, and 7B in more than one population. These results will be used in genomic selection models to improve freezing tolerance in PNW wheat.

We analyzed gene expression data for epigenetic effects of exposure to cold in the cultivar Norstar. One version of Norstar was induced to flower with the usual vernalization (exposure to cold) and the other was induced to flower only with longer daylengths. We identified 19 genes with expression differences and are following up on this research in year 3.

Impact

- The data from these cold tolerance trials was published in the seed buyers guide so that farmers can select winter wheat varieties that are less sensitive to winter kill.
- Our results from screening the regional nurseries, and screening breeding lines has been used by winter wheat breeders to select for resistance to winter injury.
- Varieties released from the WSU winter wheat breeding program have consistently excellent cold tolerance and this tolerance has been maintained because of testing using the procedures developed by this project.
- Because of the high correlation between our artificial screening trial and winter survival in the field, we can incorporate better cold tolerance into our early generation breeding lines.
- We have identified molecular markers that are being used by breeders to select for winter survival.

Freeze Survival Of Top Acreage Winter Varieties in WSU Cereal Variety Testing program, 2016-2020.								
Ranking Based On 2020 Variety Acreage Survey								
	Percent Survival	95% LCL	95% UCL			Percent Survival	95% LCL	95% UCL
Soft White Wheat					Club Wheat			
UI Magic CL+	27%	9%	44%		ARS Crescent	65%	47%	82%
Curiosity CL+	80%	63%	98%		Bruehl	54%	37%	72%
Norwest Duet	54%	37%	72%		Pritchett	56%	38%	73%
Otto	58%	43%	73%					
Norwest Tandem	52%	32%	72%		Hard Winter Wheat			
M-press	33%	13%	53%		LCS Jet	40%	23%	58%
Mela CL+	67%	49%	84%		Keldin	64%	47%	82%
Eltan	73%	62%	84%		SY Clearstone CL2	70%	52%	87%
Resilience CL+	44%	26%	62%		LCS Rocket	14%	0%	31%
Puma	50%	32%	67%		WB 4303	75%	55%	95%
SY Dayton	26%	9%	44%					
PNW Hailey	65%	41%	90%		Checks			
LCS Art deco	26%	8%	43%		Norstar	86%	75%	97%
Jasper	32%	14%	49%		Stephens	39%	28%	50%
LCS Hulk	68%	51%	86%					
SY Assure	28%	8%	48%					
LCS Drive	23%	3%	43%					
WB 1604	42%	24%	60%					
LCS Sonic	51%	33%	68%					
Bobtail	28%	8%	49%					
SY Raptor	4%	0%	29%					
SY Ovation	17%	0%	37%					
Rosalyn	23%	5%	41%					
UI Castle CL+	30%	12%	48%					
Madsen	29%	9%	49%					
Freeze Tests were conducted under controlled conditions in the WSU Plant Growth Facility between 2016 And 2020.								
These were severe survival trials. Field survival is likely to be greater, but the survival rankings from our freezing tests have correlated with field survival over multiple years.								
The precision of Confidence Intervals varies because some lines have been tested more than others.								

WGC project number: 3019-3157
WGC project title: Evaluation And Selection For Cold Tolerance In Wheat
Project PI(s): Kimberly Garland-Campbell, Karen Sanguinet and Arron Carter.
Project initiation date: 7/1/19
Project year: Year 2

Objective	Deliverable	Progress	Timeline	Communication
1. Evaluate Washington winter wheat variety trials and the hard spring wheat trials.	Ratings for freezing tolerance for commonly grown and new winter wheat cultivars and hard spring cultivars and breeding lines.	The Washington Cereal Variety Trial Winter wheat nurseries were evaluated for cold tolerance in artificial screening trials.	June 2019 - June 2022.	Presentation at grower meetings, Wheat commission meetings, Wheat Life and Research Review. Published on WSU small grains Web-site
2. Evaluate cold tolerance of new breeding lines in US regional nurseries in order to identify germplasm to use in crossing for better winter survival.	Ratings for freezing tolerance for advanced wheat germplasm from the US that can be used as new sources of cold tolerance for the PNW.	The Western Regional Winter wheat nurseries was evaluated for freezing tolerance in artificial screening trials.	June 2019 - June 2022.	Email results to regional nursery cooperators and publish on regional nursery web sites.
3. Evaluate cold tolerance of advanced breeding lines contributed by regional winter wheat breeding programs, including the WSU and USDA-ARS wheat breeding programs.	Ratings for breeding lines contributed by regional wheat breeders that will facilitate their selection decisions.	We evaluated freezing tolerance for 748 breeding lines from the Wsu Winter wheat and the USDA club wheat breeding programs and reported the data to the breeders. These data were used for selection.	June 2019 - June 2022.	Presentation at Research Review. Peer reviewed publications. Direct communication with wheat breeders.
4. Evaluate cold tolerance of F3-F5 (early generation) wheat populations that are segregating for cold tolerance and select resistant progeny.	Selections made for cold tolerance in early populations.	This was not accomplished due to less testing during the pandemic but we did release Castella club wheat in 2019, which was selected using this strategy.	June 2019 - June 2022.	Presentation at Research Review. Peer reviewed publications. Direct communication with wheat breeders.
5. Identify genes controlling cold hardiness in winter wheat.	New molecular markers and genomic selection indices for cold tolerance in PNW winter wheat and Hard red spring wheat.	We analyzed several mapping populations and identified or confirmed consistent QTLs for markers on the group 1, 4, 5, and 7 chromosomes that are being used for marker assisted selection for cold tolerance.	June 2019 - June 2022.	Presentation at Research Review. Peer reviewed publications. Direct communication with wheat breeders.

Project #: 3043-3327

Final Report Year: 2 of 2 (maximum of 3 year funding cycle)

Title: Hessian Fly Management: An Emerging Research Issue in Wheat

Researchers: **Laura Lavine, Mike Pumphrey**

Cooperators: *Arash Rashed, Arron Carter, Kim Garland-Campbell*

Executive summary: The Hessian fly *Mayetiola destructor* is an emerging economic threat to wheat grown in the inland Pacific Northwest. While screening for Hessian fly for wheat improvement has been funded by the Washington growers for several years (Pumphrey, Bosque-Pérez, & Rashed), it is critical to have new research on insect management practices for Hessian fly in Washington state based on its emergence as a new economic threat. The overall goal of this project is to increase the profitability and sustainability of Washington wheat-based cropping systems via evidence-based insect management decisions. Our specific goal is to provide new biological and ecological information on this important insect pest that will lead to improved Hessian fly management. We will accomplish this (1) providing a comprehensive review of known environmental factors contributing to Hessian fly outbreaks in the inland Northwest through surveys and collaboration with scientists, growers, and extension specialists and (2) focus specifically on genetic virulence of Hessian fly to new wheat germplasm development in several new and current genetic lines including Louise to precise recommendations for management.

Accomplishments since July 1, 2019 when this project was funded. Entomology M.S. student, Daniel Gallegos has sampled Hessian Fly from many locations including variety trial test sites in eastern Washington. Sampling has been done by hand sampling for pupae and with pheromone traps in collaboration with Ric Wesselman and Dan Maxfield from Syngenta. Sampling data is being analyzed and compared with sampling data from the summers of 2019 and 2020.

Daniel has set up a Hessian Fly wheat screening program at WSU in the older part of the CAHNRS Plant Growth Facility modeled on the facility at the University of Idaho so that we can increase the capacity of genetic lines screened.

Daniel has drafted a “What Fly is in my Grain; a visual key to identifying Hessian fly and look-alikes in PNW grains” that will be submitted for peer review in early 2021. In addition, in collaboration with Tavin Schneider (MS student in Pumphrey lab) and Samuel Prather (PhD student in Pumphrey lab), we have drafted an extension bulletin that will be submitted for peer review titled “Managing Hessian fly (*Mayetiola destructor* Say) in Washington and the greater PNW in early 2021. This extension bulletin specifically describes and provides information on management practices to control Hessian Fly that are currently available.

Impact:

Hessian fly resistance in the inland Northwest is valued from \$45 to \$104 per acre based on a study led by Dick Smiley at Oregon State University. Applying these values, a very conservative Washington state-wide loss estimate without resistant varieties is over \$10,000,000 per year, not including lower-level losses to winter wheat crops. Hessian fly infestations are widespread through the state every year and sampling with pheromone traps produces hundreds to thousands of flies at all locations sampled.

Hessian fly is largely controlled through genetic resistance maintained by expert screening of germplasm and by farmer adoption of resistant varieties. Typical insect pest management regimens for Hessian fly rely on prevention measures. From 2016-2018, we've seen heavy infestations at more sites, with heavier pressure than has been seen in over ten years or more. While newly released WSU varieties Glee, Alum, Chet, Seahawk, Tekoa, and Ryan are Hessian fly resistant due to the Hessian fly screening program funded by Washington growers, this work not only needs to continue, but the insect can and does adapt to resistant varieties. Therefore, additional research on Hessian fly population genetics and Hessian fly virulence is critical for successful management now and in the future.

No measurable impact has yet been shown in this project in the most recent funding cycle as we are setting up the foundational work for Hessian fly screening at WSU as we show in our output and outcomes table below.

Outputs and Outcomes:

A. Progress:

Objective	Deliverable	Progress
1: Comprehensive review of PNW Hessian fly biology	Published literature review of updated Hessian Fly biology; new extension bulletin published.	Literature has been reviewed; manuscript will be written in summer 2021; Visual key to identification of HF and look-alikes drafted
2: Field collections of HF; screen against varieties in greenhouse	MS grad student; HF screening at UI and at WSU (new).	HF screening at WSU in place. Screening has started and will continue using HF pupae collected in summer 2020 and from Univ of Idaho HF colonies.

C. Timeline:

Objective	Timeline
1: Comprehensive review of PNW Hessian fly biology	Summer 2021 with extension bulletin submitted to peer review by March 1, 2020
2: Field collections of HF; screen against varieties in greenhouse	Jan-June HF screening at WSU and UI; Wheat Life article after August 2021

D. Communication:

Objective	Communication
1: Comprehensive review of PNW Hessian fly biology	Presentations at virtual Field Days; peer reviewed extension bulletin
2: Field collections of HF; screen against varieties in greenhouse	Presentations at Field Days; peer reviewed scientific publication to be prepared; article for Wheat Life

What the Fly is in my Grain?

A visual key to identifying Hessian fly and look-alikes in PNW grains



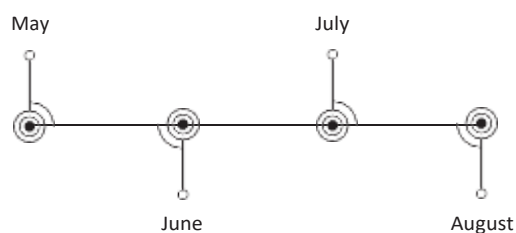
Department of
Entomology
WASHINGTON STATE UNIVERSITY



(c) Scott Bauer, USDA Agricultural Research Service

Hessian fly (*Mayetiola destructor*)

Period of infestation



Control

- Resistant wheat
- Host planteradication
- Crop rotation
- Management of stubble
- Chemical control

Damage

- Attacks wheat



(c) Dennis Schotzko, University of Idaho



(c) Samuel Prather



Department of

Entomology

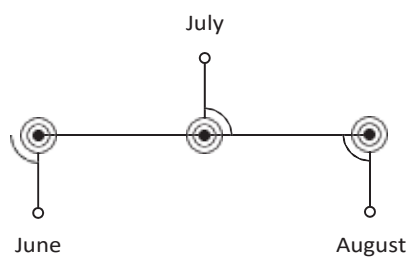
Resistant vs. susceptible wheat lines under heavy Hessian fly pressure (Susceptible line is center, resistant lines are on both sides). Photo (c) Samuel Prather



(c) Giles San Martin

Wheat midge (*Sitodiplosis mosellana*)

Period of infestation



Control

- Early planting
- Crop rotation
- Resistant wheat
- Biological control
- Chemical control

Damage

- Attacks wheat
- Each larva is capable of reducing grain size by 30-50%



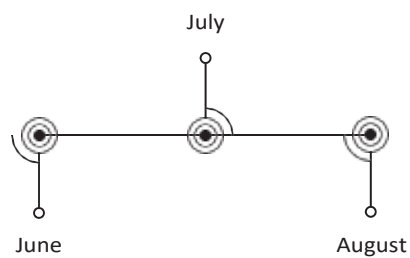
(c) Diana Roberts



(c) M. Pratheepa

Asian rice gall midge (*Orseolia oryzae*)

Period of infestation



Control

- Resistant wheat
- Host plant eradication
- Crop rotation
- Biological control

Damage

- Attacks rice - one of the worst pests of rice



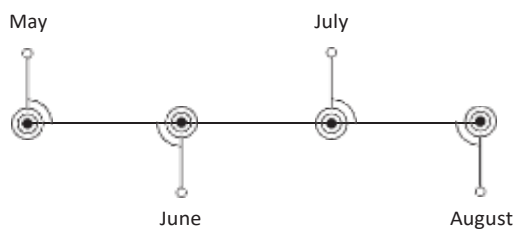
Source unknown



(c) Janet Graham

Wheat stem maggot (*Mermomyza* spp.)

Period of infestation



Control

- Chemical control

Damage

- Attacks wheat - more severe in southern US



(c) University of California



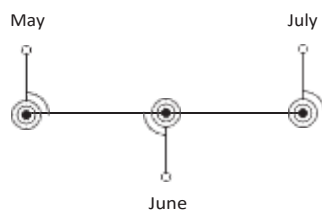
(c) M. Pratheepa



(c) Robert Peterson

Wheat stem sawfly (*Cephus cinctus* Norton)

Period of infestation



Control

- Planting trap crops
- Planting wheat in large blocks
- Resistant wheat

Damage

- Attacks wheat



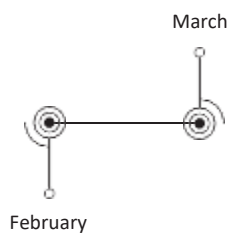
(c) Robert Peterson



(c) Ken Gray Insect Image Collection

Wheat strawworm (*Harmolita grandis*)

Period of infestation



Damage

- Attacks wheat - not a severe pest in PNW

Control

- Management of stubble

Managing Hessian fly (*Mayetiola destructor* (Say)) in Washington and the greater PNW

Authors: Tavin Schneider, Daniel Gallegos, Samuel Prather, Michael Pumphrey, Laura Lavine

Introduction

Hessian fly (*Mayetiola destructor* [Say]) is one of the most damaging insect pests of wheat around the world. Hessian flies originated in Southwest Asia, in the same regions that many of today's economically important cereal crops did (Stuart et al., 2007). In the United States, Hessian fly damage amounts to over \$100 million annually. In Washington state, studies have found that losses in susceptible varieties can reduce profits by \$40-\$100/ acre (Smiley et al., 2004; Bassi et al., 2019).



Figure 1: Hessian fly puparia in the crowns of potted wheat seedlings. Heavy infestation will split the stems of young plants, leading to lodging. Photo by Tavin Schneider.

Fly Lifecycle

The Hessian fly inflicts its damage through larval feeding on basal leaf tissue in wheat and related species. The lifecycle begins when female flies lay tiny, orange eggs on the upper surface of a young leaf (Figure 5). Three to four days after being laid, the eggs hatch into small, red larvae (Stuart et al., 2007). Larvae crawl down the inside of the leaf and into the crown of the plant or leaf nodes, where they use their mandibles to probe for feeding sites. When the insect feeds on the plant, saliva interacts with the wheat triggering the wheat to grow new tissues that will feed the growing larva and take nutrients away from the wheat itself. If the

wheat is susceptible to the Hessian fly, the larvae will feed for the next 10-20 days, providing the only sustenance during the pest's lifetime (Figure 4, 5; Stewart et al., 2007; Harris et al., 2010).



Figure 2: A full grown fly on the tip of a wheat leaf. Photo by Scott Bauer, USDA ARS.



Figure 4: Multiple fly larva (small translucent white granules) inside a susceptible wheat tiller. Photo by Samuel Prather



Figure 3: Resistant vs. susceptible wheat lines under heavy Hessian fly pressure (Susceptible line is center, resistant lines are on both sides). Photo by Samuel Prather

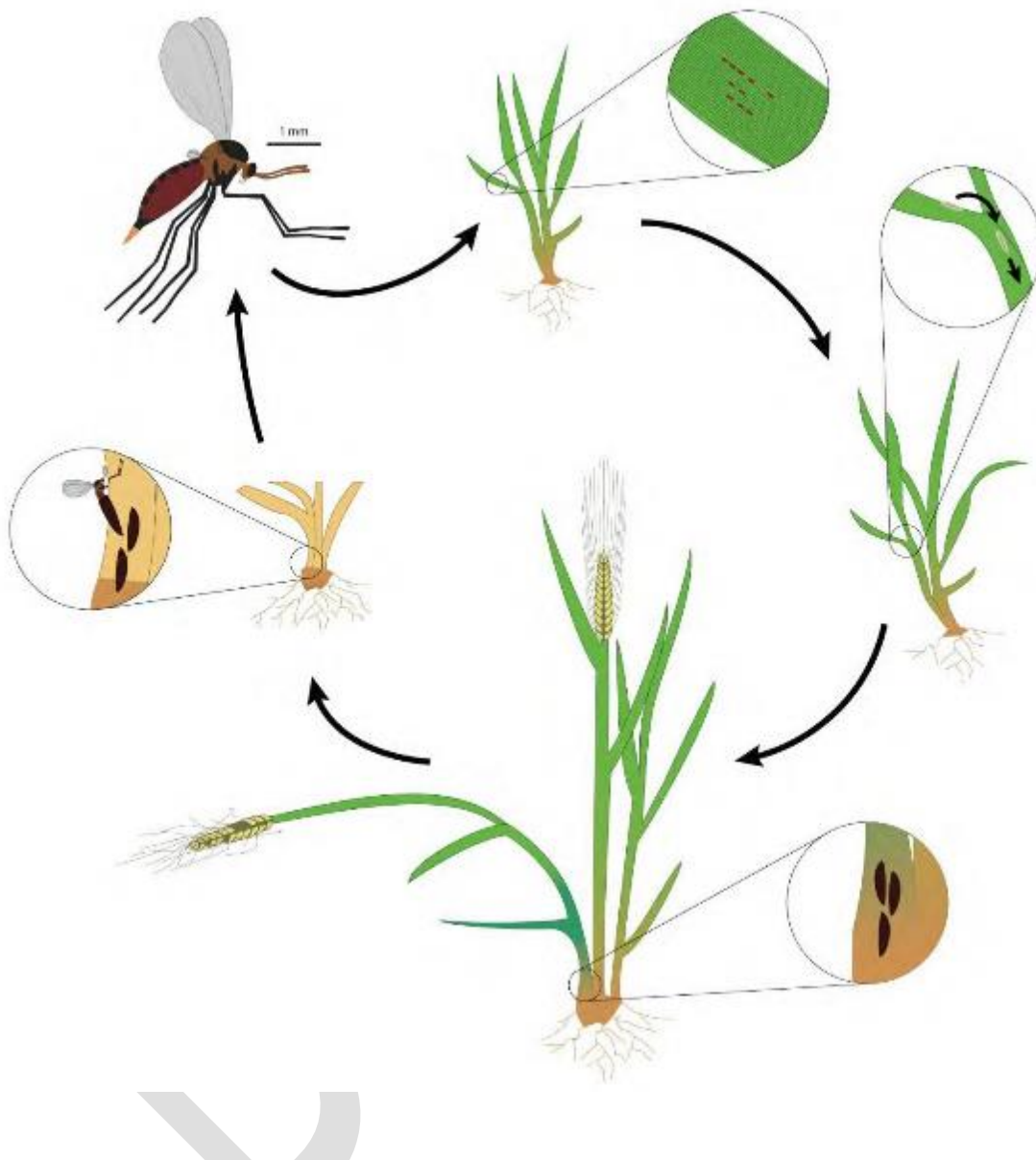


Figure 5: Complete Hessian fly life cycle, from egg laying to adult emergence. Adult female lays eggs between grooves of leaves of young plants (top), from which red larvae hatch and crawl down leaf to stem (right). As larvae grow and mature, they change in color from red to translucent white. If infestation is successful, larvae feed during first and second instars then transition to 'flaxseed' pupae near the crown (bottom). Symptoms of infestation include dead or malformed tillers, lodged stems, and a blue tint in leaves and stem (bottom). Pupae can overwinter even in wheat stubble after harvest, then emerge under optimal conditions to repeat the cycle (left and top left). Graphic by Daniel Gallegos.

After the feeding period, larvae enter a state referred to as flaxseed or puparia (Schmid et al., 2018). During this stage, the larvae are encased by a shiny, brown cover that protects

them while they mature into adult flies (Figures 1 and 5). Hessian flies spend the winter and hot, dry periods during the summer in the pupal stage, where they are protected within plant material. Depending on weather conditions, either flies will emerge from the flaxseed in the same year of being hatched, or they will overwinter within the flaxseed in wheat stubble. Hessian flies rely on high humidity levels and temperatures around 50– 79°F, with an optimum temperature of 70°F to survive (Flanders et al., 2013; Schmid et al., 2018). In the Pacific Northwest, it is more common for spring infestation to occur due to predominantly spring rain patterns, after over-wintering pupae emerge from flaxseeds, mate, and lay eggs on wheat seedlings. However, infestation and feeding can occur whenever green tissue is available and weather conditions are favorable, which is why most states experience multiple infestation events each year (Smiley et al., 2004; Flanders et al., 2013). Field observations in Washington in recent years have demonstrated potential for fall infestation of winter wheat planted during periods of fall precipitation with suitable temperatures.

Plant Damage and Crop Loss

Hessian fly larvae feeding on plant tissue results in long-term effects. Initial feeding damage is seen as darkened leaf tissue, stunted growth, and tillers with bent stems that lie on the ground (Figure 1 and 3). Long-term effects are seen through delayed and variable maturity, yield loss, lodging, and test weight reductions, which can translate to lower grain grades and dockages in elevator prices. Intense feeding can result in the death of seedlings and complete crop loss. Many infected plants will try to compensate for infestation by producing secondary tillers, which may also appear weak and dark green. If the secondary tiller avoids infestation, the plant continues to grow and stops supporting the primary tiller. Reduced wheat

competition may also lead to problematic weed management, and lodging and grain shriveling may increase volunteer seeding.

Hessian fly Control

Integrated pest management is required for Hessian fly management nationwide and in Washington state. Control methods include resistant wheat varieties, fly-free dates, host plant removal, and chemical control used in combination.

Hessian fly Resistance Genes

Resistant cultivars of wheat play a major role in mitigating the damage of Hessian fly and are an environmentally sustainable and cost-effective solution. In addition, the drawbacks and requirements associated with other control methods allow resistance genes to be among the most efficacious control option. When compatible with fly biotypes, Hessian fly resistance (HFR) genes inhibit larvae from establishing feeding sites on wheat and other host species (Figure 6). The presence of most HFR genes does not have a negative effect on wheat yields and quality parameters, so they are an attractive option to producers.

To date, 37 resistance genes have been identified; they are labeled as “*H*”, for Hessian fly, followed by a number or other designation, including *H1-H36* and *Hdic*. (Zhao et al., 2020). Many have been successfully incorporated into wheat varieties, making resistant wheat relatively easy to acquire. Hessian fly can overcome heavily used resistance genes 7-10 years after their introduction if varieties with the same HFR gene are grown year after year (Ratcliffe et al., 1994). The development of new cultivars that are effective against this pest is a continuous challenge for wheat breeders. Rotation of resistance genes in wheat varieties along

with an integrated pest management program designed for Hessian fly is vital to keep Hessian fly populations from reaching economic injury levels.

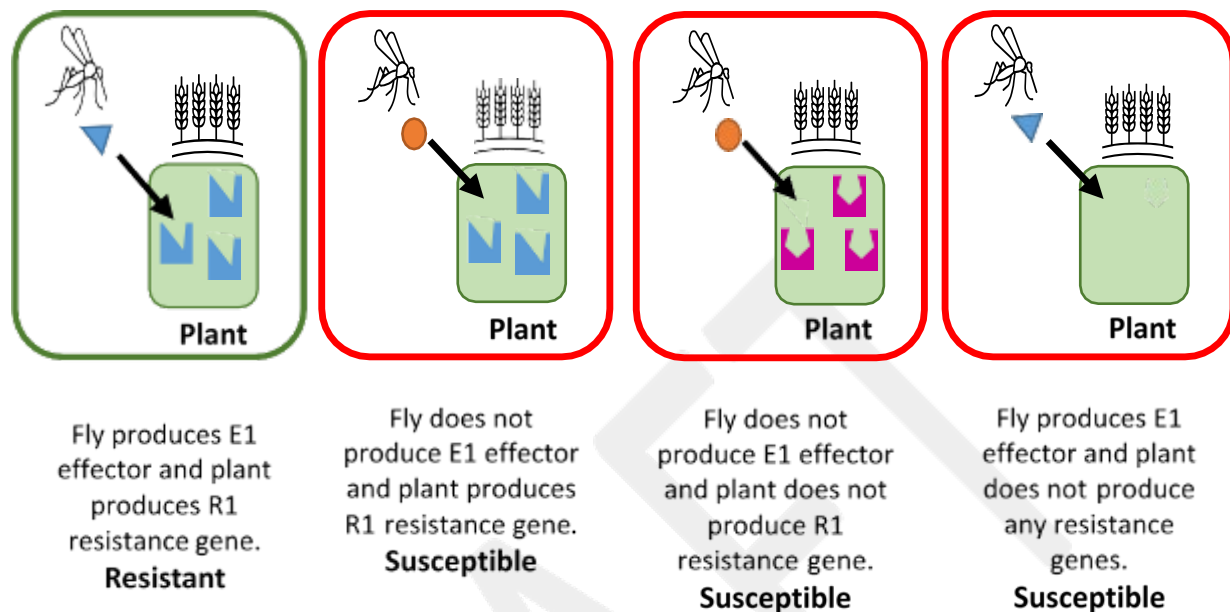


Figure 6: The interactions between Hessian fly effectors and wheat resistance genes. For all scenarios, it is possible for both the Hessian fly and the wheat plant to produce many versions of effectors and resistance proteins, respectively. Only one matching effector/resistance gene pair is needed to trigger resistance in the plant (green box). Without a matching pair, the larvae will be able to establish a feeding site (red boxes). Figure by Tavin Schneider.

Fly-free Dates:

The most basic control method in parts of the US involves adherence to fly-free dates, which are aimed at minimizing the number of larvae that infest in the fall on winter wheat crops. These dates depend on air temperatures being cool enough and humidity levels being low enough to limit Hessian fly activity. Fly-free dates are traditional options for control of fall infestation in winter wheat but have not proven to be as effective of a control measure for spring wheat. Fly-free dates are not commonly practiced in the PNW because fall infestation levels are normally minimal and cold weather damage to wheat is a much greater concern.

Following recommended fly-free dates may reduce infestation risks from other pathogens, but the delayed planting can lead to increased cold-weather damage of wheat seedlings.

Host Plant Removal:

Wheat is the main host for Hessian fly, but barley, triticale, durum, and rye can also be hosts (Harris et al., 2010). Removal of the host plant, by crop rotation, destruction of stubble, and/or volunteer plant eradication, is another widely recommended method for reducing Hessian fly infestation severity. Wild grass families including the relatives of wheatgrasses (*Elymus*), wildryes (*Elymus*), and barleys (*Hordeum*) have been found to host the insect as well (Ziess et al., 1993).

Crop Rotation:

Rotation of crops allows for a disturbance in the host plant cycle by restricting susceptible grasses from being planted in the same field for consecutive years. Preventing wheat from growing on wheat stubble dramatically reduces the availability of plants for female flies to lay their eggs on. However, neighboring fields, volunteer plants, and other grass species can harbor and spread the insects. Schmid et al. (2018) noted that flies could be distributed by wind over distances of a few kilometers, which enforces the notion that fly control is a community-wide effort.

Management of stubble:

Managing the stubble in neighboring fields and control of volunteer species around field borders can aid in the reduction of the number of insects present. Studies have found that Hessian fly infestation has become much more intense since the widespread adoption of no-till farming, which allows fly pupa to overwinter in stubble (Flanders et al., 2013). Elimination of

stubble through fall tillage has been found to reduce fly emergence by up to 70%. One downside to this approach is that intense plowing has negative impacts on soil health, erosion, and soil water conservation. Burning of stubble is ineffective for Hessian fly control because the flaxseed resides below soil surface levels, where it is protected from fire damage (Flanders et al., 2013). Removal of straw through baling is not advised because it has the potential to transfer Hessian flies from one area to another. Grass hay and wheat straw that is destined for export to Asian countries is treated with fumigation before shipping in order to prevent the spread of these insects (Yokoyama, 2011). Although mainly used for shipping efficiency, hay compression can also reduce survival of Hessian fly puparia (Yokoyama, 2011).

Chemical Control:

Insecticides, in the form of seed treatments and foliar application, can serve as a control method for fly infestation with moderate to low efficacy. Seed treatments protect against feeding insects for 1-2 months after planting (Howell et al., 2017; Schmid et al., 2018). The most popular choice for seed treatments are neonicotinoids, which are favored due to their long-lasting residuals and low application rates. Neonicotinoid seed treatment options include clothianidin, imidacloprid, and thiamethoxam. Recent discoveries in fungal-based seed treatments may provide another option for control of stem-feeding insects, such as Hessian fly (Jaronoski and Reddy, 2018).

Foliar insecticides have minimal fly control, difficult application timing, negative effects on beneficial insects, and can be expensive, making them a less appealing management option. Foliar applied insecticides may be used along with or in place of seed treatments but have serious limitations because of the Hessian fly life cycle. Sprays must be applied when eggs are

present on leaf surfaces or before larvae establish feeding sites within the plant, making timing of the spray critical (Flanders et al., 2013). The microscopic size of Hessian fly eggs makes them challenging to detect, which results in timing the applications around the most susceptible life stage very difficult. Additionally, adult flies do not all emerge at the same time, but rather throughout the wheat growing season, making damage ongoing. A single application of insecticides may reduce total fly populations by hindering one wave of hatchings, but one application of foliar insecticide does not provide control of populations throughout the entire growing season. Precipitation, wind, and temperature all affect field access and insecticide efficacy and thus may prevent farmers from making precisely timed applications.

Hessian fly and Washington Wheat

There is very little published research about Hessian flies in Washington, which furthers the need for identification of current resistance genes, implementation of additional resistance genes, and a modern understanding of the Hessian fly populations within the state and surrounding areas.

History and distribution:

The first record of the Hessian fly in Washington is disputed, with one report claiming Lewisville, Clarke County, Washington as the site of first detection of the fly in 1899, while Benson 1918 claimed that “the dreaded Hessian fly made its appearance in the state this year,” 1918, in Kent, King County, Washington (Cordley, 1900; Benson, 1918). In either case, by 1925 the fly had caused significant damage to wheat fields throughout western Washington, occurring from the Columbia River to near the southern end of the Puget Sound district in King County (Benson, 1918; Rockwood and Reeher, 1933). This was at a time when Hessian fly was

causing an average annual loss of 10% to wheat in the United States, with particularly destructive seasons resulting in losses in excess of 25% to 50% (Benson, 1918).

While the Hessian fly was known to occur throughout western Washington, ranging from the Columbia River to the Canadian border, by 1932, it was not known to occur in eastern Washington until its discovery there in 1977 (Rockwood and Reeher, 1933; Pike et al., 1978). The reason for this is twofold: virtually no records at all exist on the fly in Washington state from 1933 to 1978; and the common conception was that the fly was not as detrimental in Washington as it was in other areas of the United States during this time period (Rockwood and Reeher, 1933; Pike et al., 1978).

Reaction to resistant wheat:

A 1983 publication remains the most comprehensive overview of Hessian fly in Washington state (Pike et al., 1983). It includes the most recent distribution map of the fly, although an updated distribution map is needed.

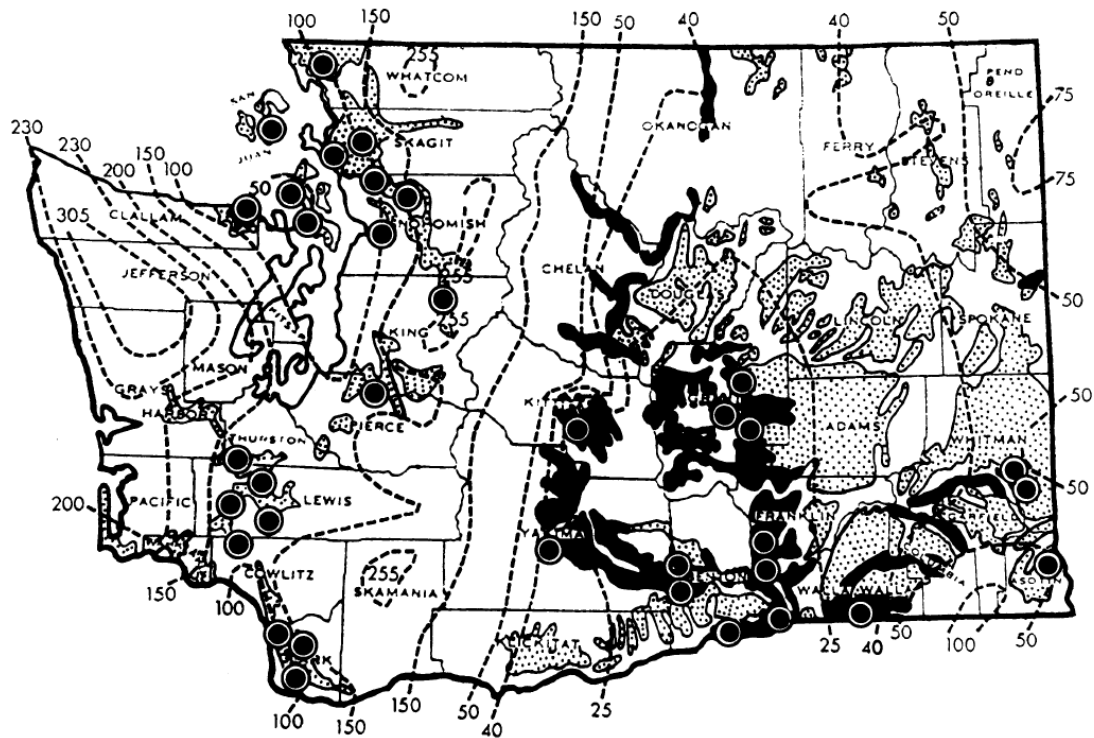


Fig. 1. Distribution of Hessian fly in Washington, 1977–1981, indicated by white-bordered black circles. Irrigated croplands shown in solid black; nonirrigated croplands in dotted pattern; average annual precipitation in cm. Cropland features after Carlson and Otis (1978), precipitation after Fisher (1941).

Previous studies in the PNW have focused on characterizing Hessian fly populations by region, but more recent research has favored classification of flies based on their ability to overcome resistant wheat varieties (Ratcliffe et al., 2009; Carter et al., 2014). Virulence, or the ability of a fly to overcome a resistance gene and thus infect a host, can often be established in fly populations within ten years of deploying wheat cultivars carrying resistance genes in a given area. When the same wheat cultivars carrying resistance genes are grown on more acres every year, fly virulence is expected to increase and with it, increased economic injury. In Washington, most genetic resistance is held within spring wheat cultivars since spring wheat is more often infested than winter wheat (Smiley et al., 2004; Table 1). However, this does not

mean that winter wheat is not infected by Hessian fly and that management can be disregarded. In fact, Pike et al. (1983) noted the heavy fall infestation was documented in XXXX area. Young wheat seedlings are most susceptible to infection, and in winter wheat, this occurs in the fall. Damaging levels of fly emergence in the fall is rare, because the dry weather and cool temperatures limit fly emergence. Irrigated winter wheat acres or years with unusually high early fall precipitation represent a higher risk. In the spring, when flies emerge from the flaxseeds, winter wheat has established multiple nodes and tillers. Stem nodes prevent movement of larvae into the basal feeding areas, and multiple tillers allow for infestation severity to be reduced. Secondary and tertiary tillers can be infected, but the yield loss isn't normally large enough to see benefits from management strategies.

Class	Name	What else can be done?
Hard Red Spring	Alum	SY Selway
	Chet	WB 9668
	Glee	Net CL+
	Hollis	Kelse
	AP Renegade	
Hard White Spring	WB 7202 CLP	
Soft White Spring	Diva	Whit
	Ryan	WB 1035CL+
	Seahawk	Louise
	Tekoa	WB 6341
	WB 6121	
Winter Club	ARS Castella	
Hard Red Winter	Scorpio	

integrate resistance mechanisms to avoid crop loss and to reduce spring infestation rates.

Table 1: Currently available Hessian fly resistant wheat varieties. Spring club wheat, a small market class of spring wheat, also lacks resistance in currently available varieties, which is one of the growers' deterrents for growing this class of wheat (Allen, 2014). Current winter wheat

breeders at WSU are working to incorporate resistance genes into the germplasm. Only a few resistant winter varieties are available now, but with focused efforts, more are expected to be released in the future.

Summary:

- Spring wheat is the main host for this pest, but management in winter wheat can reduce spring infestation levels.
- Hessian flies can be observed on wheat leaves as tiny orange eggs, or in the crown of wheat plants as white larvae or hard brown puparia.
- The most effective ways to manage for Hessian fly damage include removing susceptible host plants, utilizing seed treatments, and planting resistant cultivars. A multi-faceted approach is highly recommended.
 - Seed treatment options for fly control include clothianidin, imidacloprid, and thiamethoxam.
 - Several Hessian fly resistant genes have been deployed in spring wheat varieties. For more information please visit smallgrains.wsu.edu, where individual cultivar responses to Hessian fly can be found using the Variety Selection Tool.
- For more information:
 - The Pacific Northwest Conservation Tillage Handbook discusses Hessian fly management in reduced or no-till systems.
 - The Pacific Northwest Pest Management Handbook's section on Hessian fly describes different pesticide options.

- WSU's Variety Selection Tool is useful for identifying resistant cultivars in different growing regions.
- Extension documents from many other states go into further detail on management, identification, and economic assessments.

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

Project #: 4150-1227

Progress Report Year: 2 of 3

Title: Extension Education for Wheat and Barley Growers

Cooperators: Drew Lyon, Rachel Bomberger, David Brown, Paul Carter, David Crowder, Aaron Esser, Randy Fortenbery, Isaac Madsen, Timothy Murray, Clark Neely, Haiying Tao, Stephen Van Vleet, and Dale Whaley

Executive summary: New resources were added to the Wheat and Small Grains website in 2020. We started our first blog, Weeders of the West, with authors from WSU, Oregon State University, and the University of Idaho. A new story is posted every other week and authors respond to comments posted to the blog. Two new quizzes were added to the website: 1) the Insect & Insect Damage ID Quiz, and 2) the Soil Nutrient ID Quiz. A new PNW Extension publication (PNW754) on best management practices for managing herbicide resistance was added to the website. Other new Extension publications included revisions to PNW660 (feral rye) and 668 (downy brome). The results from the 2020 cereal variety testing program were added to the website and the Variety Selection Tool. The 2019 WSU Weed Control Report was posted as were two new Weed ID quizzes. Twenty-six new episodes of the WSU Wheat Beat Podcast were posted in 2020, a new episode every other week. There were also 49 new Timely Topics posted. The 2020 Wheat Academy was cancelled as a result of the COVID-19 pandemic.

Vacant positions on the Extension Dryland Cropping Systems Team remained a problem in 2020. The COVID-19 pandemic resulted in a canceled search for the cropping systems position in Walla Walla County, and the Spokane County Extension position was not refilled. Rachel Bomberger resigned her position in the Plant Diagnostic Clinic in December 2020. We hope that position will get refilled soon. Paul Carter announced his retirement in early January 2021, which will leave counties in southeast Washington with no agronomic expertise. The Team will enter 2021 three county positions down from where it was in 2018.

Impact: The Wheat and Small Grains website continues to be an important source of information for many people. For the 11-month period of January through November 2020, the site had 63,155 sessions with 34,808 unique users; this compares to 49,175 sessions and 36,002 unique users for the same period in 2019, and 42,484 sessions and 29,001 unique users in 2018. There were 114,379 pageviews, which is up from the 88,727 pageviews in 2019. We have 992 subscribers to our listserv, 838 followers on Twitter, and 297 followers on Facebook. The majority of the sessions from the US (41,780) were initiated from the state of Washington (16,490). We recorded 26 episodes of the WSU Wheat Beat Podcast in 2020. From January through November, the podcast had a total of 8,757 plays. Weeders of the West, a blog focused on weed science issues was initiated on March 10, 2020. Through November, the blog had 2,721 pageviews and 75 people are subscribed to the blog listserv.

WGC project number: 4150-1227 (FY 2019-2022)

WGC project title: Extension Education for Wheat and Barley Growers

Project PI(s): Drew Lyon

Project initiation date: July 1, 2019

Project year (X of 3-yr cycle): 2

Objective	Deliverable	Progress	Timeline	Communication
Add new resources to the Wheat and Small Grains website, including new publications, decision support tools and calculators, videos, and quizzes.	New and updated Extension publications, decision support tools and calculators, videos, and quizzes will be developed. Decision support tools and videos will be prioritized. An article will be written annually for Wheat Life magazine on our Extension activities.	"Resistant weed strategies from Down Under" was published in the 2020 August/September issue of Wheat Life magazine. New resources added to the website included the following: Weeders of the West Blog, an insect & insect damage ID quiz, a nutrient ID quiz, a new PNW Extension publications (PNW754), two major revisions of PNW Extension publications (PNW660 and PNW668), the 2019 WSU Weed Control Report, and the 2020 Variety Testing Data.	This will be an ongoing process throughout the duration of the project.	The development of new resources were shared with growers through Timely Topic posts on the Wheat and Small Grains website, news releases, including an annual article in Wheat Life magazine, and at various education events held throughout 2020.
Provide Timely Topics and podcast episodes on a regular and frequent basis.	We will post at least one Timely Topic every other week and one podcast episode in the alternate weeks so that we have at least one new item on the home page every week.	Twenty-six new episodes of the WSU Wheat Beat Podcast were posted in 2020; one every other week. Forty-nine Timely Topics were posted in 2020. Twenty-one articles were posted to the Weeders of the West Blog.	The proposed schedule will be initiated at the start of the funding cycle and continue throughout the duration of the project.	Timely Topics, Wheat Beat Podcast episodes, and Weeders of the West articles were posted to the home page of the Wheat and Small Grains website.
Develop a new one-day educational program along the lines of the Wheat Academy that can be held outside of Pullman.	A new one-day educational program held each year in at least one location outside of Pullman.	No progress was made on this objective in 2020.	Planning for the new educational program will begin in 2020, with delivery of the first program in 2021 or 2022.	Information on the new educational event will be communicated to growers and crop consultants through Timely Topic posts and in future advertisements for the program, including advertising in Wheat Life.
Respond to issues of concern to the wheat industry with educational programming that is timely and relevant.	In-depth educational programs, publications, articles, videos, and decision support tools as called for by the particular issue.	Herbicide resistance remained an issue of concern in 2020. The Weeders of the West Blog was started in March and posts on herbicide resistance are common. A new PNW Extension publication (PNW754) on best management practices for managing herbicide resistance was published. A Timely Topic in April addressing a late spring freeze event received 1,598 pageviews.	This will be an ongoing process throughout the duration of the project.	Educational resources and programming developed to address issues of concern were shared with growers through Timely Topic posts on the Wheat and Small Grains website and at education events held in 2020.

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

Project #: 3184

Title: Breeder Quality Trials

Cooperators: Kim Garland-Campbell, Arron Carter, Mike Pumphrey, Clark Neely

Executive summary: A new program commenced in FY 2019/20 to ‘pre-screen’ experimental breeding lines before they are entered into the WSU Wheat Variety Trials. This pre-screening was aimed at increasing the likelihood that newly released soft white wheat varieties meet industry standards before gaining substantial acreage and influencing the overall quality of the grain being exported from the Inland PNW. Quality data generated from this trial will be analyzed using the standard *t*-Score and the results (and interpretation) returned to the breeder. The data can also be included in the analysis used for the *Preferred Variety* brochure. The check variety will be drawn from the adjoining Variety Testing nursery.

Impact: Being a new program, we are not able to evaluate the impact of the project. However, we anticipate that the project will provide value to growers in two significant ways: First, it documents and highlights the quality of varieties so that breeders –public and private –are aware of the importance of quality and will hopefully include quality in their selection and variety release decisions.

Outputs and Outcomes: Following is a summary of experimental lines evaluated under this program (harvest 2020): There were 19 SWW lines submitted and they were evaluated at six locations (there were three samples missing from Dayton). The SWW lines were submitted by WSU (Dr. Arron Carter) and AgriPro. There were three SWS lines submitted and were evaluated from seven locations (one was missing from Fairfield). The SWS lines were submitted by Westbred and AgriPro. In total there were 125 samples evaluated.

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

Project #: 4721

Title: Quality of Varieties & Pre-release Lines: Genotype & Environment-“G&E” Study

Cooperators: Kim Garland-Campbell, Arron Carter, Mike Pumphrey, Clark Neely

Executive summary: The 2020 harvest sample analysis is roughly half done; the project is on-going with the most recent project covering the third of three years. As in previous years, all quality data were/will be analyzed using the *t*-Score statistic. The quality *t*-Scores for each soft white winter, club, soft white spring and club, hard red winter, hard red spring, and hard white winter and spring varieties are summarized using ‘Grain’, ‘Milling’, ‘End-Product’, and ‘Overall’ Scores. Varieties in each market class/sub-class are then ranked by the Overall Score. All varieties and advanced breeding lines with three or more years of data are included in the final listing.

Using these results and analyses, the WWQL works closely with the WGC to develop the, “*Preferred WHEAT VARIETIES for Washington based on end-use quality*” each year with annual updates. Completion of the variety rankings in February represents the first significant accomplishment each year. We coordinate variety classification with Oregon and Idaho cereal chemists.

Impact: This ‘G&E’ project provides value to growers in two significant ways: First, it documents and highlights the quality of varieties so that growers are aware of the importance of quality and will hopefully include quality in their seed-buying decisions. Data are objective “head-to-head” results on Private and Public varieties. Secondly, the data generated by the G&E study supports in a major way the analysis of new breeding lines and the WSU Variety Release process. This program is also highly visible such that good end-use quality is reinforced as a priority in both private and public breeding programs throughout the region.

Outputs and Outcomes: Following are recent advanced lines and released varieties that were supported with complete end-use quality analyses:

ALUM	HRS
ARROWHEAD	HRW
ARS09X492-6CBW	CLUB
ARS14DH1122-26	CLUB

ARS14DH1122-44	CLUB
ARSX09500-14CBW	CLUB
ARSX09500-17CBW	CLUB
ARSX12015-68CBW	CLUB
ARSX12097-8D	CLUB
ARSX12099-158	CLUB
ASSURE	SWW
BOBTAIL	SWW
BRUEHL	CLUB
BRUNDAGE96	SWW
BRUNEAU	SWW
CASTELLA	CLUB
CHET	HRS
CLEARSTONE	HRW
COMMAND	SWW
CRESCENT	CLUB
CURIOSITY	SWW
DAYN	HWS
DEVOTE	SWW
DUET	SWW
EARL	HWW
FARNUM	HRW
FUSION	HRS
GLEE	HRS
GUNSIGHT	HRS
HULK	SWW
INCLINE	HRW
JASPER	SWW
JD	CLUB
JET	HRW
KELDIN	HRW
KELSE	HRS
LOUISE	SWS
MADSEN	SWW
MAGIC	SWW
MELA	SWW
MELBA	CLUB
MONARCH	HWW
NORWEST553	HRW
ORCF102	SWW
OTTO	SWW
PIRANHA	SWW
PRITCHETT	CLUB

PUMA	SWW
PURL	SWW
RESILIENCE	SWW
ROSALYN	SWW
RYAN	SWS
SCORPIO	HRW
SEAHAWK	SWS
SELBU2	SWW
SEQUOIA	HRW
SOCKEYE	SWW
SPRINTER	HRW
STEPHENS	SWW
STINGRAY	SWW
TANDEM	SWW
WA8290	SWW
WA8293	SWW
WA8307	SWW
WA8309	HRW
WA8310	HRW
WA8312	CLUB
WA8317	SWW
WA8318	HRW
WA8319	SWW
WA8321	SWS
WA8323	SWS
WA8325	CLUB
WA8330	
WA8335	SWW
WA8336	SWW
WA8337	SWW
WA8342	HRS
WA8343	HRS
WA8344	HRS
WB528	SWW
WB9668	HRS
WHETSTONE	HRW
XERPHA	SWW

Preferred Wheat Varieties 2020 following page

SOFT WHITE WINTER

VI Frost	LCS/UI	MD
Bobtail	OSU	MD
UI Castle CL+	UI	MD
Brundage96	UI	MD
Kaseberg	OSU	MD
LCS Shine	LCS	MD
Jasper	WSU	MD
Bruneau	UI	MD
LCS Ghost	LCS	MD
UI Palouse CL+	UI	MD
Nixon	OSU	MD
Puma	WSU	MD
UI WSU Huffman	UI	MD
ARS-Selbu	ARS	D
VI Bulldog	LCS/UI	D
SY Command	AP/SY	D
LCS Biancor	LCS	D
UI Magic CL+	UI	D
Devote	WSU	D
Mary	OSU	D
LCS Shark	LCS	D
SY Ovation	AP/SY	D
LCS Drive	LCS	D
Skiles	OSU	D
WB 523	WB	D
UI Sparrow	UI	D
Eltan	WSU	D
SY Dayton	AP/SY	D
SY Raptor	AP/SY	D
Norwest Duet	OSU/LCS	D
Otto	WSU	D
WB-528	WB	D
Resilience CL+	WSU	D
Stephens	OSU	D
ORCF101	OSU	D
LCS Hulk	LCS	A
SY Assure	AP/SY	A
Purl	WSU	A
ORCF103	OSU	A
LCS Artdeco	LCS	A
Madsen	ARS	A
WB1604	WB	A
Mela CL+	WSU	A
Curiosity CL+	WSU	A
WB-1070CL	WB	A

WB1529	WB	A
WB-1066CL	WB	A
WB 456	WB	A
WB1376CLP	WB	LD
Xerpha	WSU	LD
SY Banks	AP/SY	LD
WB1783	WB	LD

HARD RED WINTER

WB4623CLP	WB	MD
Whetstone	AP/SY	MD
UI SRG	UI	MD
Norwest 553	OSU	D
LCS Rocket	LCS	D
SY Clearstone	AP/SY	D
AP503 CL2	AP/SY	D
Farnum	WSU	D
LCS Evina	LCS	D
Sequoia	WSU	D
LCS Jet	LCS	A
Keldin	WB	A
LCS Zoom	LCS	A
Esperia	Societa Produttori Sementi Spa	A
SY Touchstone	AP/SY	LD
Residence	Cebeco	UCS
Estica	Cebeco	UCS
Symphony	Tanio Tech	UCS

HARD RED SPRING

Hollis	WSU	MD
SY605 CL	AP/SY	MD
Alum	WSU	MD
SY Coho	AP/SY	MD
SY Selway	AP/SY	MD
Glee	WSU	MD
Chet	WSU	MD
LCS Luna	LCS	MD
Net CL+	WSU	D
LCS Iron	LCS	D
WB9411	WB	D
WB9668	WB	D
WB9229	WB	D
Kelse	WSU	D
Jefferson	UI	D
AP Renegade	AP/SY	D
SY Gunsight	AP/SY	D

SOFT WHITE SPRING

Tekoa	WSU	
UI Stone	UI	
Diva	WSU	
WB6341	WB	
Louise	WSU	
SY Saltese	AP/SY	
Alturas	UI	
Ryan	WSU	
Whit	WSU	
Seahawk	WSU	
Babe	WSU	
WB6121	WB	
WB-1035CL+	WB	

CLUB

ARS Castella	ARS	
ARS Crescent	ARS	
Cara	ARS	
ARS Pritchett	ARS	
Bruehl	WSU	

SPRING CLUB

Melba	WSU	
JD	WSU	

HARD WHITE WINTER*

UI Silver	UI	
Earl	WSU	

HARD WHITE SPRING*

UI Platinum	UI	
WB Hartline	WB	
Dayn	WSU	

ABBREVIATIONS

AP/SY	AgriPro/2
ARS	Agricultural Research
LCS	Limagrain Cereals
OSU	Oregon State University
UI	University of Idaho
WB	WestBred/16

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

Project #: 4722

Progress Report Year: *3 of 3*

Title: Supplemental Support for Assessing the Quality of Washington Wheat Breeding Samples

Cooperators: Kim Garland-Campbell, Arron Carter, Mike Pumphrey

Executive summary: This WGC support provides for about 3 months of additional technician time. The additional work is devoted to evaluating breeder samples for quality from early October through mid-January. During this period, spring wheat samples are given priority over winter wheat samples. The aim is to coordinate with the WSU Wheat Quality Program, and complete as many analyses as possible before spring wheat planting decisions are made in early February. In this way, the spring wheat program is made more efficient because inferior quality lines are not planted and grown. The standing goal for WSU winter wheat breeding lines is to complete as many as possible before June 1. Milling and baking evaluations of the 2019-Crop were completed and 2019-Crop testing is well under way at the Western Wheat Quality Lab.

Outputs and Outcomes: We provide breeders with SKCS single kernel size, weight, and hardness, and the variability (SD) of each; grain protein, test weight, flour yield, break flour yield, milling score, flour ash and protein, dough mixing time and type, dough water absorption, Solvent Retention Capacity (SRC) Water, Lactic Acid, Sucrose and Carbonate; SDS Sedimentation, cookie diameter and score, bread volume and score, sponge cake volume, and RVA (Rapid Visco Analyzer) peak pasting viscosity or Flour Swelling Volume (FSV) (RVA and FSV are for starch quality).

Impact: This work contributes directly to WSU and ARS variety development and release. New varieties need to be fully evaluated for end-use quality so that our customers can purchase predictable, high quality Washington wheat.

WGC project number:	4722			
WGC project title:	Supplemental Support for Assessing the Quality of Washington Wheat Breeding Samples			
Project PI(s):	Craig F. Morris and Doug Engle			
Project initiation date:	1-Jul-18			
Project year:	3			
Objective	Deliverable	Progress	Timeline	Communication
Complete spring wheat samples	Full mill & bake data delivered to breeder by early Feb.	will be reported; progress on last year's crop is on track	Starts at harvest when samples come in, ends with completion of last nursery	Data delivered directly to breeder; dialogue may ensue as to interpretation,
Complete winter wheat samples	Full mill & bake data delivered to breeder by early June	will be reported; progress on last year's crop is on track	Starts at harvest when samples come in, ends with completion of last nursery	Data delivered directly to breeder; dialogue may ensue as to interpretation,

Final Report

Project #: 3682

Progress Report Year: 2 of 2

Title: Control of Eyespot and Cephalosporium Stripe in Winter Wheat

Cooperators: **T. D. Murray, Plant Pathologist**
A. Carter, Crop & Soil Sciences, WSU
K. Garland-Campbell, USDA-ARS

Executive summary: Variety trials for eyespot and Cephalosporium stripe were conducted in 2018-19 and 2019-20. A total of 102 two new varieties and advanced lines were evaluated for resistance to eyespot and tolerance to Cephalosporium stripe in inoculated field trials. Data from these plots is used to update disease ratings in the Washington State Crop Improvement Association Seed Buyers Guide and the WSU Extension Small Grains variety selection tool.

Five growth chamber experiments were conducted to map eyespot disease resistance genes in Madsen and Cappelle-Desprez populations to determine whether the same genes control resistance to both species of the eyespot pathogen. Phenotyping and genotyping were completed, data analysis is nearly done, and a manuscript is in progress. In collaboration with colleagues in China, resistance to both species of cereal cyst nematode (CCN) was mapped in the same Madsen population and demonstrated that it carries two different genes, one each to *H. avenae* and *H. filipjevi*, both derived from VPM-1, the source of eyespot resistance. That work was published and available to breeders.

Two field experiments were conducted to determine the effect of new seed treatment fungicides and the effect of foliar fungicides on eyespot. Seed treatment fungicides did not reduce disease or improve yield over the untreated control; however, one of the foliar fungicide treatments resulted in significantly less disease and increased yield compared to the untreated control. If registered, it would be a new active ingredient for use in eyespot control.

Impact: Cephalosporium stripe and eyespot continue to be significant yield-limiting diseases for winter wheat production. Nearly all public and private breeding programs in the PNW are addressing these diseases because resistant/tolerant varieties are the most effective way to limit their impact. This project is the only place where all new varieties and advanced breeding lines are evaluated side-by-side for their reaction to eyespot and Cephalosporium stripe. The data we generate are shared with wheat breeders to support variety release and growers at variety testing field tours, online at the WSU Extension Small Grains website, and is used to provide ratings in the WSCIA seed buyer's guide and the WSU Small Grains Variety Selection tool for use by growers in making variety selection decisions.

The gene present in Madsen is the primary source of resistance in all PNW eyespot-resistant varieties and understanding its genetic control will insure it remains effective. We suspect differences in effectiveness among resistant varieties may be the result of minor genes that have not been identified and/or differences in resistance to the two eyespot fungi. Identifying minor genes affecting eyespot resistance and molecular markers for them will allow breeders to develop new varieties with more effective eyespot resistance. Screening wild relatives of wheat for new sources of eyespot resistance is an important long-term goal.

Results:

2019-20 Eyespot Variety Trial Plant Pathology Farm, Pullman, WA Dr. Tim Murray

Name	Disease Index	Rating	Name	Disease Index	Rating
LCS Blackjack	12.6	1.4	Appleby CL+	56.0	6.1
Rosalyn	14.9	1.6	WA 8305 CL+	56.4	6.2
LWW16-71088	16.7	1.8	IDO1906 HWW	56.7	6.2
OR2x2 CL+	23.8	2.6	UIL 17-6268 CL+	57.0	6.2
WA 8293	26.1	2.9	WA 8316	57.6	6.3
Resilience CL+	26.2	2.9	IDO1808 SWW	60.2	6.6
Madsen (resistant check)	26.8	2.9	UIL 17-6451 CL+	60.9	6.7
Nixon	30.4	3.3	Farnum	61.1	6.7
VI Bulldog	31.7	3.5	IDO1810 SWW	64.3	7.0
Puma	32.1	3.5	UIL 17-6834 CL+	64.7	7.1
ARS09X492-6CBW	32.4	3.5	Sequoia	65.1	7.1
Pritchett	33.8	3.7	UI Bronze Jade	65.7	7.2
08PN030-3	34.4	3.8	Scorpio	66.0	7.2
VI Frost	34.6	3.8	SY Dayton	66.9	7.3
WA 8271 (Devote)	37.0	4.1	aMaze	68.0	7.4
WA 8307	39.8	4.4	LCS Rocket	69.4	7.6
UI Sparrow	40.2	4.4	Eltan (susceptible check)	70.7	7.7
WA 8289	42.2	4.6	WB4311	70.9	7.8
IDO1608 HRW	43.8	4.8	WB1529	71.7	7.8
Stingray CL+	44.0	4.8	WA 8310	72.0	7.9
YS-201	44.0	4.8	Keldin	73.8	8.1
WA 8309	44.8	4.9	AP Redeye	73.9	8.1
WA 8287	45.2	5.0	IDO1806 HWW	76.8	8.4
ARS-Castella	47.2	5.2	LCS Ghost	77.2	8.5
WA 8306 CL+	49.0	5.4	IDO1607 HRW	77.6	8.5
WA 8308	50.5	5.5	IDO1506 HWW	79.5	8.7
WA 8290	51.1	5.6	WB4394	80.6	8.8
WA 8317	54.3	5.9	WA 8318 CL+	81.6	8.9
Purl	54.6	6.0	LCS Zoom	83.0	9.1
11PN044#84	54.6	6.0	LSD 5%	14.3	

Values represent the mean of three replicates. Plants were removed and evaluated for disease incidence and severity on 6/23/20 to 6/29/20. Eyespot severity was determined by evaluating stem bases, 1 to 2 internodes above the crown, for symptom severity using a 0 to 4 scale where 0 = no visual symptoms, 1, 2 and 3 = up to 25, 50 and 75% of the stem circumference colonized by a lesion(s), respectively, and a 4 = a stem with a lesion girdling the base. Disease severity is the weighted mean of all evaluated stems and incidence is the percentage of stems with symptoms. Disease index was calculated by multiplying disease incidence by disease severity and dividing by four and ranges from 0 to 100. The disease Index was significantly different among varieties ($P > F = 0.0001$).

Rating reflects the degree of resistance and ranges from 1 to 9, where 1 = highly resistant and 9 = highly susceptible.

2019-2020 Eyespot Foliar Fungicide Trial
Plant Pathology Farm, Pullman, WA
Dr. Tim Murray

Treatment	Rate, fl. oz/ac	Mean			Yield bu/ac	Bushel wt lbs/bu
		DI%	DS	Index		
Control		93.7	3.2	75.1	92.1	58.5
Miravis Ace	13.7	57.5	2.5	35.8	104.2	59.0
Miravis Ace	7.0	73.3	2.7	49.0	111.9	59.6
Tilt + Telaris	4 + 10	92.8	3.1	73.0	92.0	58.3
Aprovia Ace	7.0	93.3	3.2	75.4	90.3	58.3
Trivapro	9.4	96.1	3.3	78.1	91.1	58.0
Trivapro	13.7	96.4	3.3	79.9	92.2	57.8
Aprovia Ace	13.7	96.2	3.4	81.9	91.2	58.3
LSD 5%		10.5	0.2	9.7	12.2	1.0

The plot was planted 19 September 2019 with a 1:1 mixture of ‘Eltan’ and ‘Hill 81’ winter wheat cultivars. Inoculation with a suspension of conidia of both eyespot pathogens was made 6 November 2019. Plots were sampled during the first two weeks of June by digging plants from three locations in each plot and combining them. Tops were removed and outer leaf sheaths removed. Visual ratings of lesion severity were made using a 0 to 4 scale where: 0 = no visual symptoms, 1, 2 and 3 = up to 25, 50 and 75% of the stem circumference colonized by a lesion(s), respectively, and a 4 = a stem with a lesion girdling the base. Disease severity is the weighted mean of all evaluated stems and incidence is the percentage of stems with symptoms. Disease index is calculated by multiplying disease incidence by disease severity and dividing by four and ranges from 0 to 100. Plots were harvested on 8 August 2020 with a Wintersteiger NurseryMaster plot combine.

Publications:

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- Carter, A.H., K.A. Balow, G.B. Shelton, A.B. Burke, K.E. Hagemeyer, A. Stowe, J. Worapong, R.W. Higginbotham, X.M. Chen, D.A. Engle, T.D. Murray, and C.F. Morris. 2020. Registration of 'Stingray CL+' Soft White Winter Wheat. *J. Plant Registrations* [SUBMITTED 15 July 2020].
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- Carter, A.H., K.A. Balow, G.B. Shelton, A.B. Burke, K.E. Hagemeyer, A. Stowe, J. Worapong, R.W. Higginbotham, X.M. Chen, D.A. Engle, T.D. Murray, and C.F. Morris. 2020. Registration of 'Devote' Soft White Winter Wheat. *J. Plant Registrations* [IN PRESS 15 July 2020].
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- Kruse, E.B., K.L.E. Klos, J. Marshall, T.D. Murray, B.P. Ward, and A.H. Carter. 2019. Evaluating Selection of a Quantitative Trait: Snow Mold Tolerance in Winter Wheat. *Agrosystems, Geosciences & Environment* 2:190059.
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Presentations:

- Identification and Management of Winter Wheat Diseases. 2020 Last Chance Pesticide Seminar, Asotin, WA, December 8, 2020.
- Identification and Management of Winter Wheat Diseases. Spokane Ag Show, Spokane, WA, February 6, 2020.

Identification and Management of Winter Wheat Diseases. 2020 Nutrien Grower Meeting, Spokane, WA, January 31, 2020.

Effect of Soil Acidity on Wheat Diseases. Soil Acidity Workshop, WSU Farmers Network, Pullman, WA, January 16, 2020.

Identification and Management of Winter Wheat Diseases. Walla Walla Cereal Seminar, Walla Walla, WA, January 14, 2020.

Diagnosing and Managing Diseases of Wheat. WSU Wheat Academy, Pullman, WA, December 10, 2019.

Identification and Management of Winter Wheat Diseases. Far West Agricultural Association Annual Meeting, Pasco, WA, December 9, 2019.

Biology and Management of Winter Wheat Diseases. Washington State Crop Improvement Association Annual Meeting, Spokane, WA, November 18, 2019.

Wheat Production and Disease Constraints in Washington. 111 Summer Workshop, Jilin Agricultural University, Jilin, China, August 23, 2019.

Climate Change and its Impact on Winter Wheat Diseases in the Northwestern U.S.A., 111 Summer Workshop, Jilin Agricultural University, Jilin, China, August 25, 2019.

Climate Change and its Impact on Wheat Diseases, 2nd International Conference on Northeast Asia Biodiversity, Baishan, China, August 27, 2019.

Update on Snow Mold, Stripe Rust, and Eyespot Diseases of Wheat. Highline Grain Growers Annual Meeting, Waterville, WA, February 25, 2019.

Soil Acidity – Its causes and Impacts on Wheat Diseases. Management Matter for Soil Health, WSU Farmers Network, Pullman, WA, February 21, 2019.

Biology and Management of Winter Wheat Diseases. Spokane Ag Expo, Spokane, WA, February 5, 2019.

Update on Snow Mold, Stripe Rust, and Eyespot Diseases of Wheat, and Hessian Fly. 2019 Syngenta Cereal & Pulse School, Spokane, WA, February 4, 2019.

Cultural Management of Soil-borne Plant Diseases. Management Matters for Soil Health, WSU Farmers Network, Pullman, WA, January 22, 2019.

Biology and Management of Winter Wheat Diseases. Walla Walla Cereal Seminar, Walla Walla, WA, January 15, 2019.

WGC project number: 3682
WGC project title: Control of Eyespot and Cephalosporium Stripe in Winter Wheat
Project PI(s): T. Murray, A. Carter, K. Garland-Campbell
Project initiation date: 7/1/19
Project year (X of 3-yr cycle): 2 of 2

Objective	Deliverable	Progress	Timeline	Communication
1. Evaluate advanced breeding lines and new varieties for resistance to eyespot and Cephalosporium stripe in field plots	Provide unbiased data on the resistance reactions of advanced selections and new varieties to eyespot and Cephalosporium stripe.	<p>2020: Forty-two winter wheat cultivars and breeding lines were evaluated for their resistance/tolerance to eyespot and Cephalosporium in inoculated field trials in June 2019. Another 60 lines were planted in September for evaluation in 2020.</p> <p>2021: Sixty winter wheat cultivars and breeding lines were evaluated for resistance/tolerance to eyespot and Cephalosporium stripe in inoculated field trials in June 2020.</p>	<p>2020: This was the third year of testing in collaboration with the WSU Variety Testing program and WSU Winter Wheat Breeding.</p> <p>2021: This was the fourth and final year of testing varieties and advanced lines for resistance to eyespot and Cephalosporium stripe in collaboration with the WSU Variety Testing program.</p>	Results from these plots are presented at field days, variety plot tours, and other talks to grower and industry groups, and available online at the Extension Small Grains Team website. Data are used to update variety ratings in the Washington State Crop Improvement Seed Buyer's Guide, the WSU Extension Small Grains Variety Selection tool, and published online in Plant Disease Management Reports so they are available to the larger wheat research community.
2. Screen wild wheat relatives and other genetic populations to identify and map potential new eyespot resistance genes	Identify potential new eyespot resistance genes for use by breeders to improve effectiveness of resistant varieties.	<p>2020: Conducted two experiments to screen 135 lines from a Cappelle Desprez x Whetstone population for eyespot resistance; one study each with Oy and Oa was completed and data have been summarized. A third experiment was setup in December for the second round of screening. DNA was collected from all lines and submitted for genotyping. We anticipate completing the screening experiments in late spring and then follow with analysis of the data.</p> <p>2021: Three growth chamber experiments were conducted to determine whether the same genes control resistance to both species of the eyespot pathogen. Phenotyping and genotyping was completed, data analysis is nearly done and a manuscript is in progress.</p>	<p>2020: We anticipate beginning work on populations of the wheat relative <i>Dasypyrum villosum</i> in collaboration with Chinese wheat breeders in early summer or fall 2020.</p> <p>2021: Seed of the <i>Dasypyrum</i> population was not received as a result of COVID-19 restrictions on research in China. We hope to receive the seed to evaluate in the future.</p>	Results of this research will be shared with breeders, presented at field days, variety testing plot tours, and other talks to grower and industry groups. Data also will be published in appropriate scientific journals.
3. Evaluate fungicides currently registered for eyespot control for effectiveness and determine the potential for development of fungicide resistance	Provide data that will help growers and field consultants make decisions about whether and which fungicide to use in controlling eyespot by testing fungicides registered for eyespot control in multiple locations in eastern WA.	<p>2020: Two field trials were planted in September 2019; one for the evaluation of eight seed treatments and another for spring-applied foliar fungicides for eyespot control.</p> <p>2021: Disease and yield evaluation of two fungicide trials, one for seed treatment and the other for foliar fungicides, was completed during summer 2020</p>	2020: Fungicides will be tested annually depending on interest and support from industry. Work on resistance has not begun.	Results from this research will be presented at field days, variety plot tours, and other talks to grower and industry groups, and available online at the Extension Small Grains Team website. Results also will be published in Plant Disease Management Reports so they are available to the larger small grains pathology community.
Prepare an article for Wheat Life during the three-year project.	Prepare an article for Wheat Life during the three-year project.	2020: Management options for wheat diseases in the Inland PNW published in January Wheat Life	2020: One article published in January	

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

Project #: 4127-1605
Progress Report Year: 2 of 3
Title: Evaluation of Barley Varieties
Researcher: Clark Neely
Cooperators: Robert Brueggeman

Executive summary: Of the 12 spring barley variety trials planted in 2020, Palouse and Pullman were the only data not published due to high unexplained variability within the trial. The trials included seven feed, eight malt, two hulless food varieties, and seven experimental lines. Three private companies and two land grant breeding programs entered material into the trial. Dry conditions in March and early April allowed for most sites to be planted one to four weeks earlier than normal. Cooler temps and precipitation picked up in late April and hung around through June improving yields. All 2020 barley variety trial data was uploaded by November 25 on to the WSU Small Grains website (<http://smallgrains.wsu.edu>). The 2020 final report with additional information was posted January 5. In addition to our website and final technical report, variety performance information was delivered to barley growers and other clientele through virtual field tours, emails with preliminary results after harvest (over 200 recipients), Wheat Life article, WSCIA seed buying guide, and reports to the Washington Grain Commission. The Variety Testing Program was unable to conduct in-person field days due to COVID-19 and so virtual field days were recorded at four locations and posted on the WSU CAHNRS YouTube Channel (www.youtube.com/user/WSUCAHNRS).

Impact: Variety selection is a major decision on most farms and influences not only yield potential, but other management factors (input costs) such as insect and disease (pesticide applications), fertility, and herbicide program. The WSU spring barley VT has two important direct impacts: 1) Provides critical and unbiased data for growers to make informed decisions on which barley varieties are best adapted for their environment and management practices and 2) provides breeders, seed companies and seed dealers reliable information to make decisions regarding experimental line advancement, varietal releases/seed increases, and marketing strategies. Plant pathologists also use these trials to rate disease reactions. On average in 2020, there was an 890, 710, and 620 lb/a advantage between the highest yielding spring barley variety and the trial average in the >20", 16-20", and 12-16" precipitation zones, respectively. At \$6.35/cwt, potential additional income generated ranges from \$39 to \$57/acre. Multiplied across 71,000 acres of harvested barley, these trials have the potential to generate approximately \$3.3 million across the state.

WGC project number: 4127-1605				
WGC project title: Evaluation of Barley Varieties				
Project PI(s): Clark Neely				
Project initiation date: 07-01-2019				
Project year (X of 3-yr cycle): 2 of 3				
Objective	Deliverable	Progress	Timeline	Communication
1. Conduct representative and objective barley variety field trial evaluations at locations that represent major production areas of Washington.	12 spring barley trials; 24 entries/trial	2020 trials completed (24 entries/trial) 2021 trials in planning	Trials are planted in the spring, data results are available to growers at the end of the harvest season. Field tours in summer.	Results from the variety trials are communicated via Extension programming and are detailed under Objective #4.
2. Entries in trials will include: currently grown varieties and promising advanced breeding lines from the major public and private breeding programs in the region.	All widely grown, commercially available varieties and promising experimental lines are included in trials.	2020 barley entries: 50% public, 50% private. Every major spring barley breeding program in the PNW is actively participating in the VTP.	Entries confirmed by February 12th.	Solicit entries by January 15. Maintain positive relationship with breeding programs to ensure future participation.
3. Provide access to variety trials and harvested grain enabling other researchers and supporting projects to gather information from the trials.	Participation and ratings from other projects/ programs.	Cooperation with breeders, pathologists, seed dealers, WSCIA, other universities, and Extension. Data are used by breeders for variety release and promotional materials.	Ongoing cooperation and collaboration that fit with timelines and other listed objectives.	Disease ratings presented in seed buyers guide and variety selection tool. VTP data used for variety release and PVP applications.
4. Deliver an Extension education program to make the results and interpretation of the variety trials available to growers, the seed industry, and other clientele.	a.) Grower meetings	Attended Adams Co. Grower Meeting and PNW Farm Forum in 2020. Others cancelled due to COVID-19.	Will attend when invited	Attend in person; present results through powerpoint presentation and handouts.
	b.) Field Tours	10 in-person field days planned for 2021 (will film virtual field days if in-person not allowed); virtual field days recorded at Reardan, Dayton and Pullman in 2020.	June-July	*List of Field Days provided below; provide paper handouts of data
	c.) Email List Serv	2020 results delivered	October through December	Email list serve: Data sent to 213 members as it becomes available
	d.) Website	Up to date with 2020 data	October through December	9,950 pageview for 2019 VT data (in 2020); 8,267 pageviews for 2020 VT data;
	e.) Annual Report	All data analysis is complete; site management information and supplemental tables being added.	December	The annual report will be published as a WSU technical report online and hard copy.
	f.) WSCIA Seed Buyers Guides	Tables in preparation	January-February	Seed Buyers Guide published in January-February
	g.) Wheat Life	Spring barley VT article completed	Barley VT article submitted December 2020	Article published in Wheat Life in January each year
	h.) Variety Selection Tool (smallgrains.wsu.edu)	Selection tool updated with spring barley 2020 data.	December	The variety selection tool had 10,426 pageviews in 2020.
*Anticipated 2021 in-person field days are subject to COVID-19 restrictions. If in-person events are not possible, virtual field days will be posted online instead. Field days typically held at Almira, Dayton, Fairfield, Farmington, Lamont, Mayview, Palouse, Reardan, St. John, and Walla Walla.				

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

Project #: 4127-1604
Progress Report Year: 2 of 3
Title: Evaluation of Wheat Varieties
Researcher: Clark Neely
Cooperators: A. Carter, K. Garland-Campbell, M. Pumphrey

Executive summary: For the 2020 season, the Variety Testing Program planted 23 SWWW, 15 HRWW, 18 SWSW, and 18 HRSW trials. Of these, two SWWW, one HRWW, and one HRSW trial were lost due to resistant weed issues or high variability within the trial. Weather was mostly favorable throughout the state with at or above average yields for the majority of sites. Dry conditions in March and early April allowed for most spring sites to be planted one to four weeks earlier than normal. Cooler temps and precipitation picked up in late April and hung around through June improving yields. Dry conditions in the early fall of 2020 delayed winter wheat emergence in higher rainfall zones despite timely planting. All 2020 individual location winter data was posted by August 30 (spring wheat data was posted by October 23) on to the WSU Small Grains website (<http://smallgrains.wsu.edu>). The 2020 final report with additional information was posted January 5. 2021 Winter Field Maps were posted online November 23. The Variety Testing Program was unable to conduct in-person field days due to COVID-19 and so virtual field days were recorded at four locations and posted on the CAHNRS YouTube Channel (www.youtube.com/user/WSUCAHNRS).

Impact: With over 90% of Washington wheat planted with certified seed, variety selection is a major decision on most farms and influences not only yield potential, but other management factors (input costs) such as insect and disease control (pesticide applications), fertility, and herbicide program. The WSU variety testing program provides critical and unbiased information for growers to make informed decisions on which wheat varieties are best adapted for their environment and management practices. On average in 2020, there was an 9, 11, 7, and 11 bu/a spread between the highest yielding variety and the trial average for the SWWW, HRWW, SWSW, and HRSW trials, respectively. At \$5.56/bu, potential additional income generated ranges from \$39 to \$61/acre. Multiplied across the 1.75 million and 0.54 million acres of harvested winter and spring wheat, respectively, these variety trials have the potential to generate approximately \$103 million across the state. In addition to grower benefits, breeders, seed companies and seed dealers rely on this information to make decisions regarding experimental line advancement, varietal releases/seed increases, and marketing strategies. Samples and data generated from these trials are also critical for wheat quality testing and development of the Preferred Wheat Varieties brochure. This information is important for promoting adoption of high-quality wheat varieties and helps secure overseas markets. Collectively, 47 individual presentations went into producing the four virtual field days that received 1,393 views.

WGC project number: 4127-1604				
WGC project title: Evaluation of Wheat Varieties				
Project PI(s): Clark Neely				
Project initiation date: 07-01-2019				
Project year (X of 3-yr cycle): 2 of 3				
Objective	Deliverable	Progress	Timeline	Communication
1. Conduct representative and objective wheat variety field trial evaluations at locations that represent major production areas of Washington.	a) 24 soft winter wheat trials; 48-60 entries/trial	a) 2021 winter trials planted; 2020 results finished Collaborative trials are continuing with OSU at Eureka and Walla Walla.	Trials are planted in the spring or fall, data results are available to growers at the end of the harvest season. Field tours/virtual field days in summer.	Results from the variety trials are communicated via Extension programming and are detailed under Objective #4.
	b) 16 hard winter wheat trials: 30-36 entries/trial	b) 2021 winter trials planted; 2020 results finished		
	c) 18 soft spring wheat trials; 22 entries/trial	c) 2020 results finished		
	d) 18 hard spring wheat trials; 30 entries/trial	d) 2020 results finished; 2021 fall-planted DNS trials planted		
2. Trial entries include: currently grown varieties and advanced breeding lines from major public and private breeding programs in the region.	All widely grown, commercially available varieties and promising experimental lines are included in trials.	2020 winter trials; 52% public, 48% private. Every major breeding program in the PNW is actively participating in the VTP. 2021 winter entries, locations, and maps have been posted on the variety testing website.	2021 winter entries were confirmed by August 21st and spring entries will be confirmed by February 12th.	Solicit winter entries by August 5 and spring entries by January 15th. Maintain positive relationship with breeding programs to ensure future participation.
3. Provide access to variety trials and harvested grain enabling other researchers and supporting projects to gather information from the trials.	Participation and ratings of characteristics from other projects/ programs.	Cooperation with breeders, pathologists, entomologists, quality lab, FGIS, seed dealers, WSCIA, other universities, and Extension.	Ongoing cooperation and collaboration that fit with timelines and other listed objectives.	Quality results in preferred variety pamphlet, falling number results presented by corresponding project, disease ratings presented in seed buyers guide, VTP data used for variety release and PVP applications. All data/ratings included in variety selection tool.
4. Deliver an Extension education program to make the results and interpretation of the variety trials available to growers, the seed industry, and other clientele.	a.) Grower meetings	Attended Adams Co., Spokane Co. and PNW Farm Forum meetings (2020). Others cancelled (COVID).	Will attend when invited.	Attend in person and present results through powerpoint presentation and handouts.
	b.) Field Tours	21 planned for 2021; virtual field days will be filmed at select locations if in-person field days are not possible due to COVID-19 restrictions.	June-July	*List of Field Days provided below; provide paper handouts of data
	c.) Email List Serv	2020 results delivered	August through November	Email list serve: Data sent to 213 members as it becomes available
	d.) Website	Up to date with 2020 data	August through December	9,950 pageview for 2019 VT data (in 2020); 8,267 pageviews for 2020 VT data; 171 pageview for 2021 field maps
	e.) Annual Report	All 2020 data analysis is complete; site information and supplemental tables included in the final report that is now posted online.	December	The annual report will be published as a WSU technical report online and hard copy.
	f.) WSCIA Seed Buyers Guides	2020 tables completed and published in guide and 2021 tables in preparation.	January-February	Seed Buyers Guide published in January-February
	g.) Wheat Life	Spring VT article completed; Winter VT article to be written in May 2021	Spring VT article: January Winter VT article: May	Articles published in Wheat Life in January and May each year.
	h.) Variety Selection Tool (smallgrains.wsu.edu)	Selection tool updated with spring 2020 data. 2020 winter data to be uploaded in January 2021.	December - January	The variety selection tool had 10,426 pageviews in 2020.
*Anticipated 2021 in-person Wheat Field Days are subject to COVID-19 restrictions. If in-person events are not possible, virtual field days will be posted online instead. Field days held at Horse Heaven, Ritzville, Dusty, Connell, Lind, Harrington, St. Andrews, Eureka, Walla Walla, Dayton, Moses Lake, Creston, Almira, Reardan, Mayview, Anatone, Fairfield, St. John, Lamont, Bickleton, Farmington, Palouse.				

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

Project #: 3019-3189
Progress Report Year: *1 of 1*
Title: Weather Stations to Support Variety Testing and Wheat Research
Researcher: Clark Neely
Cooperators: David Brown, Camille Steber, Kim Garland-Campbell, Xianming Chen

Executive summary: The purpose of this project was to obtain and install Tier 2 (Atmos 41) weather stations at all variety trial locations that were greater than 5 miles from the nearest WSU AgWeatherNet (AWN) station. Data collected includes air temperature, humidity, dewpoint, wind speed and direction, precipitation, solar radiation, soil temperature, soil moisture, and leaf wetness. The original locations identified included Creston (20 mi.), Bickleton (18 mi.), Connell (15 mi.), Lamont (12 mi.), Plaza (12 mi.), Fairfield (11 mi.), Colton (11 mi.), Mayview (10 mi.), Dusty (8mi.), Endicott (6 mi.), Palouse (6 mi.), Moses Lake (6 mi.), and Reardan (5 mi.) plus Walla Walla (4 mi.) because of the sharp rainfall gradient in the area. Stations have successfully been installed at all sites except Plaza because that location is transitioning to a new cooperator. It will be installed spring of 2021 once the new cooperator is identified. All newly installed stations are now within two miles of the trial and all but three locations are less than one mile. While these stations can be moved, they are meant to be stationary for consistent historical data. Weather data is updated every 15 minutes onto the WSU AgWeatherNet website (<https://weather.wsu.edu/>). Connectivity is limited by cell phone reception and so stations at Endicott and Lamont are currently unable to update regularly to the website, but are still collecting weather data that can be harvested by hand. Eventually a directional antennae will be connected to the stations to boost signal. The VTP already has or is currently coordinating with AWN program to move some preexisting stations closer to trials at Harrington, Farmington, Anatone, St. Andrews (complete), and St. John (complete).

Impact: Short term impacts include the ability for the VTP to more efficiently plan maintenance trips based on weather conditions for locations that require significant travel time. Cooperators and nearby growers also use these stations to plan field work. 2020 in-season rainfall totals were included in the final variety testing technical report for spring trials, but not winter trials since they were not installed until halfway through the growing season (will be included in subsequent years). This information will be useful in interpreting results of the trials and will provide a historical weather database moving forward for researchers to create growth models and disease prediction models. Dr. Camille Steber regularly uses AWN weather station data to compare with falling number data and better weather data will help with our understanding of late maturity alpha amylase (LMA) occurrence in wheat.

WGC project number:	3019-3189
WGC project title:	Weather Stations to Support Variety Testing and Wheat Research
Project PI(s):	Clark Neely
Project initiation date:	July 2020
Project year (X of 3-yr cycle):	1 of 1

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

(Begin 1 page limit)

Project #:3061-7667

Progress Report Year: 1 of 3 (maximum of 3 year funding cycle)

Title: **Management of Nematode Diseases with Genetic Resistance**

Scot Hulbert, Kimberly Garland Campbell and Timothy Paulitz

Executive summary and Final Report for Year 1 of grant.

This grant was not funded for the 2nd year of this cycle. A previous 3-year summary was submitted for the previous cycle (Jan. 2019) and Year 1 of the grant (Jan. 2020). Here, we report on our progress from Jan 1, 2020 to June 30, 2020, which covers the last half of Year 1 grant. Because of COVID, activity was severely curtailed in the lab, but we did accomplish the following

- collected infested soil in Fall, 2019 to use in greenhouse screening and testing in 2020.
- continue to cross resistant/susceptible lines for RIL and NIL populations, as described in the previous report.
- we used a known *Cre* sequence to blast against the Chinese Spring genome, identified similar sequences, found SNPs near the region (this was recorded in last year's report) and developed markers for these SNPs. However, at present, without reliable *Cre* gene differential lines with single *Cre* genes, it was difficult to draw conclusions from these markers.
- started screening a winter wheat population, as part of an USDA pre-doctoral scholarship for Nikayla Strauss, a PhD student with Drs. Campbell and Paulitz. The synthetic wheat population used in this project is the D-genome Nested Association Mapping (DNAM) population. The DNAM population is the result of 8 different hybridizations of *Ae. tauschii* with a hexaploid hard white Kansas breeding line. The *Ae. tauschii* accessions were chosen for their diversity compared to the D-genome in modern wheat varieties. All Recombinant Inbred Lines (RILs) in this population have the same genetic material for the A and B genomes, varying only in genes in the D-genome. During preliminary greenhouse CCN screenings, it was discovered that lines within DNAM family U6713 have resistance to both species of CCN found in the US, *Heterodera avenae* and *Heterodera filipjevi*, especially TA1642 (Figure 1). KS05HW14 exhibited resistance to *H. avenae* but not *H. filipjevi*. Thus, this resistance would be extremely useful if incorporated into elite adapted varieties because the germplasm we are starting from is relatively well adapted.

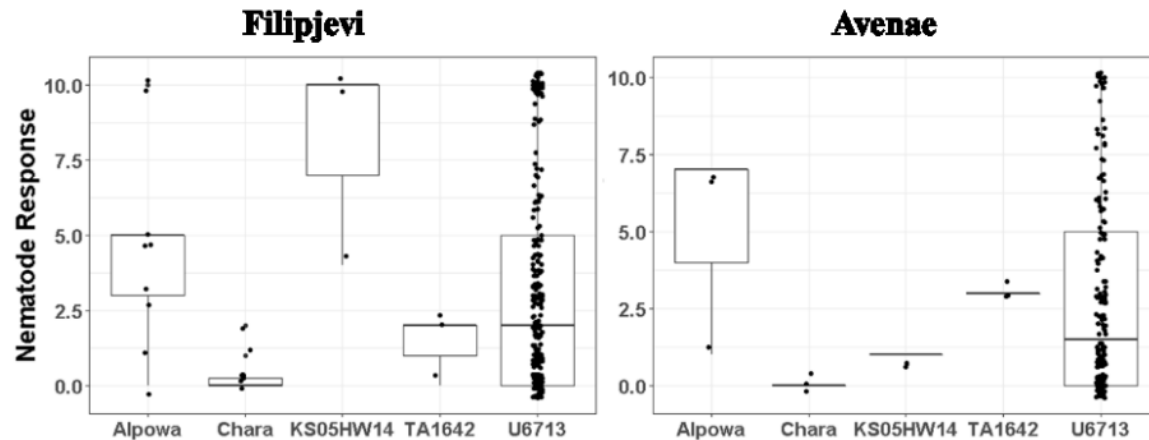


Figure 1. Results of multiple greenhouse screenings of family U6713. For these screenings, the checks are as follows: Alpowa (S), Chara (R), KS05HW14 (recurrent parent), TA1642 (*Ae. tauschii* parent of U6713 family). KS05HW14 exhibits different responses to *H. filipjevi* and *H. avenae*. The U6713 population varies in score but is skewed towards resistance.

Report from Year 1, submitted Jan. 2020.

- Over the last 2 funding cycles, we have developed a greenhouse method to screen PNW varieties and germplasm for resistance to cereal cyst nematode (CCN) which has become a problem in eastern Washington, first recognized in 2010. We have screened over 1000 lines, but are limited by finding field inoculum with high levels of the nematode. The populations in some of our field sites has declined, so we have had to locate new sources of inoculum. This resulted in less lines screened this year. Working in the greenhouse, we are constrained by the number of lines that can be screened.
- The ideal solution to replace phenotyping in the greenhouse is to develop DNA markers linked to CCN resistance genes or loci. To that end, we have developed two series of markers. The first was from mining the Chinese Spring genome and looking for sequences close to known *Cre* genes. These were converted to KASP markers which can be run in a high throughput fashion. We have identified four potential markers that show a correct reaction on a set of differentials with known *Cre* genes and resistance. The second set of SSR markers for Cre 3, 5, 8, and X were designed from the literature. But only *Cre* 3 and 8 worked, and it appears that lines in the differential set may have more than one *Cre* gene in them. Both sets of markers will now be tested on a wider range of PNW material.
- To identify new resistance genes in PNW material, we crossed known resistant varieties such as Chara and ARS Crescent and susceptible lines Alpowa, Seahawk, Bruehl, Ouyen and Louise. We are now backcrossing them to develop a mapping population which will be phenotyped, so we can locate new QTL or resistance genes.
- We imported differential lines for identification of CCN pathotypes from Turkey. We have done preliminary pathotype screening with this set of differentials, and our isolates of *H. filipjevi* do not match known races. We also made a new collection of cysts from a number of locations in Aug. 2019 and a colleague in Turkey extracted cysts to take back to Turkey to also run the pathotype test. In addition, we developed a collection of fungi from the cysts that may be potential biocontrol agents.

Impact:

- By developing molecular markers for CCN resistance genes, we can greatly enlarge the amount of germplasm and variety testing, since we will not be reliant on lengthy greenhouse testing and

dependent on field inoculum which may not be stable.

- We will continue to identify resistance sources that can be incorporated into PNW adapted varieties.

-

- What measurable impact(s) has your project had in the most recent funding cycle?

- We have a set of markers that may be useful in detecting multiple *Cre* genes, and will be tested with a wider range of PNW varieties to determine their utility.
- Identified resistance in a new mapping population of D-genotype (D-genome Nested Association Mapping (DNAM) population). These RILs developed from this population will enable us to identify the genes and move them into elite lines.

Deliverables

A growing list of resistant US and PNW varieties and lines, which can be used directly by the growers or incorporated into existing breeding programs.

A greenhouse technique that is optimized for screening more lines for the breeders

Greenhouse pot cultures of *H. avenae* and *H. filipjevi* that can be used for screening of varieties

A beginning knowledge of what *Cre* genes we may have in our backgrounds

A complete understanding of the distribution of *H. avenae* and *H. filipevi* in eastern Washington, including distribution maps.

The first description of the pathotype of *H. filipjevi* in eastern Washington

Refereed papers

Wen, N., Thompson-Manning, Y., Garland-Campbell, K. and Paulitz, T. C. 2019. Distribution of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in Eastern Washington State. Plant Disease 103:2171-2178.

Thompson-Manning, Y., Wen, N., Garland-Campbell, K. and Paulitz, T. C. 2021. Genome-wide association mapping for cereal cyst nematode resistance in Pacific Northwest wheat germplasm. In preparation

Abstracts

Wen, N., Thompson-Manning, Y., Garland-Campbell, K. and Paulitz, T. C. 2019. Distribution of cereal cyst nematodes (*Heterodera avenae* and *H. filipjevi*) in Eastern Washington State. 7th International Cereal Cyst Workshop, Nov. 2019. New Delhi, India

Presentations

Paulitz, T. C. 2019. What's New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 6, 2019

Paulitz, T. C. 2019. Nematode Diseases of Cereals. Far West Agribusiness Association, Pasco, WA. Dec 9, 2019.

Paulitz, T. C. 2020. What's New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 5, 2020

Paulitz, T. C. 2020. Nematode diseases of cereal crops. Presented to Agricultural Research Council of the Ukraine, Sept. 11, 2020.

WGC project number: 3061-7667
WGC project title:Management of nematode diseases with genetic resistance
Project PI(s): S. Hulbert, T. Paulitz, K. Campbell
Project initiation date:7/1/2019

Project year 1: 2019-2020

This report covers the last half of Project Year 1, Jan. 2020- June 30, 2020. Section in **Bold** indicates new work started not reported in previous report

Objective	Deliverable	Progress	Timeline	Communication
1. Continue to identify CCN (specifically <i>H. filipjevi</i> and <i>H. avenae</i>) resistance in wheat varieties adapted to PNW.	List of resistant US and PNW varieties and lines, knowledge of what <i>Cre</i> genes we have in our backgrounds	To date, we have screened over 1000 lines in our greenhouse assay, using infested field soil. However, our source of soil for <i>H. filipjevi</i> declined in population, but we located another hot location in Aug. 2019. We have collected soil for vernalization, to use in 2020, along with <i>H. avenae</i> soil.	Will continue greenhouse testing next year using vernalized, infested soil in the greenhouse.	Paulitz, T. C. 2019. “Root Disease Research at ARS Pullman-What’s New?” Spokane Farm Forum, Ag Expo, Feb. 26 2019. (presentation). Paulitz, T. C. 2019. Nematode Diseases of Cereals. Far West Agribusiness Association, Pasco, WA. Dec 9, 2019 (presentation) Paulitz, T. C. 2020. What’s New in Research on Soilborne Plant Pathogens. Spokane Farm Forum, Ag Expo, Spokane, Washington. Feb. 5, 2020 (presentation) Paulitz, T. C. 2020. Nematode diseases of cereal crops. Presented to Agricultural Research Council of the Ukraine, Sept. 11, 2020 (Presentation)

2. Develop more knowledge about specific resistance genes and develop molecular markers	Usable markers that can be incorporated in the breeding programs.	<p>We have made considerable progress this year. Using the <i>Cre</i> 1 sequence from the database, we mined the genome of Chinese Spring and found a number of <i>Cre</i> susceptibles. We developed KASP markers and tested them on the Australian differentials with know <i>Cre</i> gene resistance. A number of markers look good, and seem to identify both <i>Cre</i> 1 and <i>Cre</i> 3. The best is IWA3381 which will now be tested on PNW lines with know resistance. SSR markers were tested for <i>Cre</i> 3, 5, 8, and X based on published literature. The markers for <i>Cre</i> 3 and 8 worked, but in the differential collection, many lines with supposedly a single <i>Cre</i> gene also were detected with <i>Cre</i> 3 and 8. This indicates that these lines may have multiple <i>Cre</i> genes. Despite the fact that these markers seem to pick up multiple <i>Cre</i> genes, they will be useful for germplasm screening.</p> <p>We started screening a winter wheat population, as part of an NSF pre-doctoral scholarship for Nikayla Strauss, a PhD student with Drs. Campbell and Paulitz. The synthetic wheat population used in this project is the D-genome Nested Association Mapping (DNAM) population. The DNAM population is the result of 8 different hybridizations of <i>Ae. tauschii</i> with a hexaploid hard white Kansas breeding line. The <i>Ae. tauschii</i> accessions were chosen for their diversity compared to the D-genome in modern wheat varieties. All Recombinant Inbred Lines (RILs) in this population have the same genetic material for the A</p>	Markers will be tested in 2020 on PNW material with known resistance and susceptibility Delayed because of COVID	
3. Identify unknown resistance genes in adapted PNW material.	New resistance genes for cereal cyst nematode	Lines (NILs) will be developed by crossing CCN resistant lines Chara and ARS-Crescent with CCN susceptible lines Alpowa, Seahawk, Bruehl, Ouyen and Louise, then backcrossing. We have made all the initial crosses, and will continue to develop these mapping populations	Backcrosses will be made in 2020. Continuing despite COVID	
4. Determine pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i>, using differential lines being imported from Turkey.	knowledge of pathotypes of <i>H. filipjevi</i> and <i>H. avenae</i>	Collections of CNN populations were made in Aug. 2019, with a Borlaug Fellow from Turkey. We will pathotype these on our differential varieties imported from Turkey, but he also took back a collection to pathotype there. In addition, we isolated a collection of fungi from cysts to look for potential biocontrol agents	Pathotyping will be done in WA and Turkey in 2020 Delayed because of COVID	

Fusarium Grant 2018-2020

3019-3685

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 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. 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.

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**
Project initiation date: **7/1/2018**
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 A. tauschii, 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 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.	

Washington Grain Commission

Wheat and Barley Research Annual Progress Reports and Final Reports

Project #: 3019 3162

Progress Report Year: 2 of 3 (maximum of 3 year funding cycle)

Title: Improving Spring Wheat Varieties for the Pacific Northwest

Cooperators: Mike Pumphrey, Vadim Jitkov, Vic DeMacon, Sheri Rynearson, Wycliffe Nyongesa

Executive summary:

The WSU spring wheat breeding program's elite material and recently released varieties continue to be the top performers in statewide variety trials and for growers. *A new 2-gene Clearfield spring club wheat was released in 2020, Hedge Cl+.* Foundation and registered seed of hard red spring Net Cl+ was produced and sold in 2020. Each variety has very good to excellent end-use quality, which is a primary goal of our program to help maintain and increase the value of Washington wheat. *WSU soft white spring wheat varieties accounted for 92% of certified soft white spring wheat production acres in Washington in 2020.* Our newest soft white spring wheat varieties, Ryan, Seahawk, Tekoa, and Melba, have broad adaptation, superior all-around disease, grain, and agronomic traits, most desirable end-use quality, and top yield performance. They have been rapidly adopted by seed dealers and Ryan was by far the leading variety in the state with over 160,000 acres planted. Glee, Chet, and Alum are leading dryland hard red spring wheat varieties, and Net CL+ has been rapidly adopted. *WSU spring wheat varieties collectively were planted on 70% of the certified spring wheat production acres in Washington in 2020.* The consistency, broad adaptation, disease and pest resistances, sound grain traits, most desirable end-use quality, good falling numbers, and overall performance of these varieties reflects the outputs of comprehensive wheat breeding and genetics research effort supported primarily through funding from this project.

Impact:

The WSU spring wheat breeding program is in a unique position to focus on grower opportunities and challenges, large and small. We identify and develop traits, technology, germplasm, and release varieties to meet the needs of the majority of Washington producers, whether the needs are localized or widespread. We emphasize traits like stable falling numbers, Hessian fly resistance, stripe rust resistance, and aluminum tolerance, and hold the entire industry to a greater standard for yield, yield protection, and end-use quality. Our latest releases package excellent yields with superior quality and key yield protection traits. Public wheat breeding programs at WSU and across the country consistently pay back on research dollars invested. *With 70% or more of the spring wheat acres in Washington planted to WSU spring wheat varieties in 2019, growers continue to realize a substantial return on research dollars invested in this program.* The yield of our top three released soft white spring wheat varieties (Ryan, Seahawk, Tekoa) averages 107% of the top three varieties from other programs, using 5-year average data from over 60 variety trials in >12" precipitation locations from 2016-2020.

Outputs and Outcomes: File attached

WGC project number: 3019 3162

WGC project title: Improving Spring Wheat Varieties for the Pacific Northwest

Project PI(s): Mike Pumphrey

Project initiation date: 2019

Project year: 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
Develop biotic and abiotic stress tolerant, high-yielding, and high-quality hard red, soft white, club, and hard white spring wheat varieties for diverse Washington production environments.	New spring wheat varieties that are superior to existing varieties. This effort includes all four market classes of spring wheat and all precipitation regions in Washington state.	WSU released varieties generated significant positive economic impact for PNW growers in 2020 by our varieties being planted on 70% of spring wheat acres. Five year yield trial data for the top three WSU SWS wheat varieties across all locations >12" precipitation demonstrates that our market-leading varieties produced 107% of the top three varieties from other breeding programs. We had very good test plots across regions in 2020. Good data quality is fundamental to making solid selections. Our 2-gene Clearfield breeding efforts have matured, and we released a new spring club CL+ line in 2020. Our attention to stable falling numbers over the past five years has resulted in selection of superior lines for this trait.	Recurring annually	WSU Field days, Private company field days, Workshops/meetings/presentations attended/given by Pumphrey: Western Wheat Workers, WSCIA Annual Meeting, WSCIA Board, WA Grain Commission, Trade tours/international buyer groups. Annual Wheat Life contributions as requested
Improve PNW spring wheat germplasm to strengthen long-term variety development efforts/genetic gain.	Enhanced germplasm. Consistent genetic gain for many desirable traits.	Multiple stripe rust, aluminum tolerance, Hessian fly, and quality traits were selected in backcross populations for long-term parent building in 2020. A primary focus in 2020 was backcrossing new Hessian fly resistance genes into spring wheat germplasm. Extensive crossing blocks for irrigated hard red spring wheat germplasm development were also completed. Two large fall-seeded spring wheat trials were conducted in 2020 with irrigation. Backcrossing of the AXigen trait for CoAXium wheat production system was continued. We are backcrossing into both soft white and hard red spring wheat germplasm.	The payback for this work will fully be realized for many years to come as these lines continue to be crossed into existing breeding lines. We expect this effort to result in introgression of desirable variation for yield, disease resistance, and other agronomic characters.	
Discover/improve/implement scientific techniques and information to enhance current selection methods.	Current projects are development of DNA markers for useful sources of Hessian fly and stripe rust resistance, drought and heat tolerance loci, identification of superior germplasm through association mapping, screening for tolerance to aluminum, development of facultative wheat, and the development of high-throughput field phenomics selection methods.	Several specific trials and locations were again evaluated in 2020 to help long term breeding efforts. Scientific products of our efforts through multiple projects in 2020 include five publications in high-quality international scientific journals. Information from these research efforts help guide specific germplasm development efforts focused on Hessian fly, stripe rust, genomic selection, high-throughput phenotyping, association mapping, marker-assisted selection, drought tolerance, heat tolerance, yield, test weight, gluten strength, etc.	This work has short, medium, and long term goals. We are already using new DNA markers discovered through this work to improve selection for quality and pest resistance.	

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

Project #: 3163

Progress Report Year: 2 of 3 (*maximum of 3 year funding cycle*)

Title: **Greenhouse and laboratory efforts for spring wheat variety development**

Cooperators: Mike Pumphrey, Vic DeMacon, Sheri Rynearson, Wycliffe Nyongesa, Vadim Jitkov

Executive summary:

This project is integral to core efforts of the Spring Wheat Breeding program. This project provides funding to make crosses and develop breeding populations in the greenhouse, staff support for management and selection of breeding materials in the field and greenhouse, and supports/enables the most effective end-use quality selection procedures for development of superior Washington spring wheat varieties. In addition to routine early-generation grain quality selection carried out through this project, we apply DNA marker technology to elite breeding materials, and conduct research projects of direct relevance to our breeding efforts. This project also supports our two-gene Clearfield and AXigen breeding efforts, Fusarium head blight resistance gene introgression, Hessian fly resistance gene introgression, and expanded irrigated hard red spring wheat breeding efforts. Our progress in each of these areas is consistent, and these outputs shape our overall breeding efforts.

Impact:

This project is critical to the spring wheat breeding program and establishes our core breeding efforts. Program efficiency is increased by evaluating early generation lines for quality and eliminating those with poor quality characteristics before further testing. Increased testing of superior material in the field program protects resources from being used to further test lines that are inferior in terms of quality, lack adequate pest resistance, and numerous other DNA-marker selectable traits. Spring wheat varieties with complex stripe rust resistance, Hessian fly resistance, aluminum tolerance, superior end-use quality, and broad adaptation are critical for Washington wheat producers by adding millions of dollars of annual return. Over the past five years, we have released Seahawk, Tekoa, Melba, Ryan, Net Cl+, and Hedge CL+. They have been rapidly adopted by seed dealers and growers and are top-volume sellers through the Washington State Crop Improvement Association Foundation Seed program. The consistency, broad adaptation, disease and pest resistances, sound grain traits, most desirable end-use quality, good falling numbers, and overall performance of these varieties reflects the outputs of comprehensive wheat breeding and genetics research effort. Two new releases are planned for 2021 and will provide growers a top-performing Hessian fly resistant club spring wheat variety (WA8325) and a broadly adapted hard red spring wheat (WA8315) with excellent yield, disease resistance, quality, and slightly higher protein than our current top-end dryland varieties beginning in 2022.

Outputs and Outcomes: File attached

WGC project number: 3019 3163

WGC project title: Greenhouse and laboratory efforts for spring wheat variety development

Project PI(s): Mike Pumphrey

Project initiation date: 2019

Project year: 2 of 3

Objective	Deliverable	Progress	Timeline	Communication
Develop DNA markers and select breeding lines by marker-assisted selection with stripe rust resistance, Hessian fly resistance, and two-gene Clearfield™ herbicide tolerance as well as other traits when desirable.	Elite variety candidates will result, in part, due to these molecular selection activities. Many of these populations will be ideal for marker optimization, new genetic mapping studies, and potentially the basis of new competitively funded projects.	Two Wheat Life articles were written/contributed in 2020, as well as supporting other articles. Axigen trait introgression continued, and we have made BC3 materials with this new herbicide tolerance to date. We have developed new DNA markers for a previously undocumented Hessian fly resistance locus that allow us to track resistance in most of our germplasm for the first time. KASP assays have been developed. Our second backcrossed, marker-assisted selection, doubled haploid, two-gene Clearfield variety was released in 2020, in a JD background.	Activities are cyclical and occur annually throughout the normal breeding cycles.	Pumphrey attended/presented at numerous virtual WSU field days, workshops/meetings, PNW wheat Quality Council, WSCIA Annual Meeting (presentation), WSCIA Board Meetings, WA Grain Commission meetings, industry tours.
Select early-generation breeding lines with good end-use quality potential by eliminating inferior breeding lines prior to expensive and capacity-limited yield tests.	Elimination of lines with inferior end-use quality. This ensures only lines with acceptable end-use quality are tested in the field and maximizes efficiency in field operations. Current analyses include: NIR-protein, NIR-hardness, SKCS-hardness, SDS micro-sedimentation, PPO, and micro-milling.	Another year of selection was successfully completed in 2020, with approximately 2400 lines evaluated through the various quality tests. Markers for PPO and waxy alleles were validated and applied to breeding materials.	Return on investment is realized each year, since lines with poor end-use quality are not tested in expensive and capacity-limited yield tests. This allows for additional yield testing of lines with good end-use quality and more efficient variety development.	
Conduct greenhouse operations required for variety development, including crossing, doubled haploid development, generation advancement, and seedling assays such as herbicide screening, and stripe rust screening.	Lines for field testing that contain desirable and novel characteristics. This is where new varieties are born. Greenhouse operations also allow more rapid breeding cycles by advancing F1 and F5 generations every year as part of our routine breeding efforts. Seedling evaluation of stripe rust resistance and herbicide tolerance screening are also major greenhouse activities.	We continue to use the expanded greenhouse space to our advantage to increase breeding and research materials, make crosses, and conduct experiments. COVID-19 has some, but minor impact on our ability to conduct this day to day work.	Greenhouse multiplication and crossing is completed annually, including two large crossing blocks and thousands of early generation lines tested for stripe rust and herbicide tolerance.	

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

Project #: 3208

Progress Report Year: ___1___ of ___3___ (*maximum of 3 year funding cycle*)

Title: **End-Use Quality Assessment of Washington State University
Wheat Breeding Lines**

Cooperators: *Mike Pumphrey, Arron Carter, Craig F. Morris, Doug Engle*

Executive summary:

WSU spring and winter wheat variety development programs heavily emphasize selection for superior end-use quality. Quality evaluation of WSU breeding lines has been ongoing for over 50 years. Effective quality testing is essential for the recent release of new varieties from all market classes that are at or near the top of end-use quality rankings. This project supports a scientist to conduct thousands of quality tests per year for the WSU wheat breeding programs in conjunction with USDA-ARS Western Wheat Quality Laboratory efforts.

The majority of wheat from the PNW is exported to overseas markets. To maintain current markets and penetrate new markets, PNW wheat must possess quality characteristics that make it superior for use in both domestic and overseas markets. Therefore, before it is released, a new variety must be tested to determine if it is suitable for use in specific end-use products. In addition, increased competition from traditional and non-traditional export countries necessitates enhancing the end-use quality of our wheat. The loss of overseas markets would continue to cause a reduction in the demand and therefore the price of wheat, resulting in losses to Washington farmers. Washington wheat growers, as well as grain buyers and exporters, benefit from the availability of wheat varieties that require less inputs and possess superior, consistent end-use quality.

Impact:

Otto, Puma, Devote, Jasper, Sequoia, Stingray CL+, Glee, Net CL+, Chet, Alum, Seahawk, Melba, Tekoa, Ryan, are examples of top-performing WSU variety releases that are widely grown that also have very good to excellent end-use quality. One of our primary goals as public breeding programs in Washington State is to set a high-bar for end-use quality, and continue to raise that bar for long term market health. By releasing lines with superior agronomics, paired with most desirable end-use quality, we provide growers with options that put quality in the decision process, while not sacrificing yield or other agronomic and protection traits. Several of our newest varieties are preferentially sourced because of their superior end-use quality, and specific traits like gluten strength and breadmaking quality, low cadmium concentration, partial waxiness, and outstanding cookie and cracker quality. This short, medium, and long-term impact is of paramount importance to the Washington grain industry.

Outputs and Outcomes: File attached

WGC project number: 3208
WGC project title: End-Use Quality Assessment of Washington State University Wheat Breeding Lines
Project PI(s): Mike Pumphrey and Arron Carter
Project initiation date: 1-Jul-20
Project year (X of 3-yr cycle): 1 of 3 year cycle

Objective	Deliverable	Progress	Timeline	Communication
Early to late generation quality testing of WSU experimental lines to aid variety development	New spring wheat and winter wheat varieties that are superior to existing varieties. This effort includes all market classes of spring and winter wheat and all precipitation regions in Washington state.	Over 1200 breeding samples were analyzed by numerous milling and baking quality tests each year of this project. Four new wheat varieties were released in part due to this project and data in 2020. Others are planned for 2021 release.	The economic return for this work will manifest itself each breeding cycle with superior quality varieties and germplasm.	Progress will be summarized and discussed at numerous field days (>10 per year), grower meetings (~10 per year), the annual Research Review, through WSCIA meetings, Wheat Life, Variety Release Meetings, and direct communication with the WGC every year. Arron Carter participates in multiple US Wheat trade tours and we hosted many trade teams in 2019-2020.
Support genetic analysis of end-use quality to identify desirable alleles and to predict end-use quality through new genotyping methods	Improved germplasm selection procedures which translate to more efficient, cost-effective, and consistent genetic gain for end-use quality.	Multiple special milling and baking trait experiments were evaluated in 2020, including evaluation of DNA markers for PPO and a dough strength locus. Milling and baking analysis of a bi-parental winter wheat mapping population has also been completed. A genetic map of hard red spring wheat quality QTL was generated, and this work presented at the PNW Wheat Quality Council with a peer-reviewed manuscript in preparation.	The reward for this work will compound each year and will fully be realized for many years to come as these lines continue to be crossed into existing breeding lines. We expect this effort to result in routine selection of outstanding quality wheat.	

Washington Grain Commission

Wheat and Barley Research Annual Progress Reports and Final Reports

Project #: 3019 3674

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

Title: Evaluation of WSU wheat breeding lines for management of Hessian fly and development of DNA markers for resistance breeding

Cooperators: Mike Pumphrey, Arash Rashed

Executive summary:

Hessian fly (HF) infestations continue to cause significant annual yield losses in spring wheat production areas of Washington and neighboring regions of Oregon and Idaho. Hessian fly is in many ways a silent problem. Moderate infestations are not visually striking, and their occurrence is somewhat variable over space and time. Factors such as weather patterns, crop rotation, variety selection, and tillage or conservation practices can impact HF pressure. Infestation may also be a significant barrier to increased conservation tillage practices in Washington. Advanced breeding lines, new sources of resistance genes *H13*, *H26*, and two unknown resistance sources, along with winter wheat varieties were screened for Hessian fly resistance in 2020. Backcross populations were developed with four new sources of resistance, and progeny advanced to select homozygous resistant lines. Winter wheat populations and varieties were screened to introgress HF resistance into winter wheat. This project supported the screening of all new entries in WSU Variety Testing Program spring wheat trials, and approximately 80 winter wheat varieties. NEW DNA markers that allow diagnostic tracking of an undocumented spring wheat resistance source were developed.

Impact:

Spring wheat production has averaged ~30 million bushels in WA in recent years. A conservative state-wide loss estimate of 2% translates to over \$4,000,000 per year; yield loss due to HF in moderately to heavily infested areas often exceeds 25% and may be 100% in localized areas. In addition to protecting from \$45-\$104 per acre via HF resistance, improved variety development can translate to \$Millions/year in WA spring wheat farm gate value. Our recent emphasis on winter wheat is due to infestations increasingly observed in winter wheat in the region. While not as severe as spring wheat infestations, we believe the value of Hessian fly resistance in winter wheat is underestimated, and increasing.

Our most recently released soft white spring wheat varieties Seahawk, Tekoa, and Ryan, and hard red spring wheat varieties Net Cl+, Glee, Alum, and Chet, are resistant to Hessian fly because of selection activities carried out by this collaborative project. Given their broad acreage in Washington State, this represents a major economic impact to Washington farmers.

Outputs and Outcomes: attached

WGC project number: 3674
WGC project title: Evaluation of WSU wheat breeding lines for management of Hessian fly and development of DNA markers for resistance breeding
Project PI(s): Pumphrey
Project initiation date: 2018
Project year: 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
Screen WSU Spring Wheat breeding populations and advanced breeding lines for resistance to Hessian fly in the laboratory	Information on resistance of elite breeding lines on an annual basis	Over 300 spring wheat lines, 40 winter wheat varieties, numerous breeding populations, and new entries into the WSU Wheat Variety Testing Program were screened in 2020. Data has been shared with Extension personnel, through the Variety Selection Tool, WSCIA	Annually	Progress was presented by M. Pumphrey at field days, plot tours, at Wheat Research Reviews for individual states. Presentations will be made to the Washington Wheat Commission and WAWG conferences upon invitation. Progress will be reported in Wheat Life magazine and data will be recorded with nursery data.
Continue to incorporate "new" Hessian fly resistance genes into breeding lines	Improved germplasm with useful sources of Hessian fly resistance	Several backcrosses have been made to known (H13, H26) and unknown resistance gene donors, using susceptible elite line "Dayn" as the initial recipient parent. BC4-derived Dayn-Hessian fly resistant introgression lines were evaluated in yield plots and for grain quality in 2020, and elite performers used in routine breeding crosses in fall 2020. Also, JD and Melba were used to introduce four new resistance sources through backcrossing with phenotypic selection. Diagnostic DNA markers for the resistance present in Seahawk, Kelse, and some other spring wheat varieties was developed and validated in 2020.	Annually	

Washington Grain Commission Wheat and Barley Research Final Report

Project #: 13C-3019-3687

Progress Report Year: ___3___ of ___3___ (*maximum of 3 year funding cycle*)

Title: *A Genetic Arsenal for Drought Tolerance, Getting to the Root of the Problem*

Cooperators: *Karen A. Sanguinet (CSS) , Kim Garland-Campbell (USDA/ARS; CSS), Timothy Paulitz (USDA/ARS; Plant Pathology)*

Executive summary

Lignin content and accumulation in stems, leaves and roots has been linked with different stress tolerances in crop plants. Lignin confers rigidity to plant cell walls, and increases in response to drought, heavy metals, salinity, and pathogen attack. Therefore, managing overall lignin content, as well as its proportion in the roots versus shoots of crop plants is important for improved stress tolerance. Only a few studies have investigated the role of lignin in grass root systems at present. Reports on maize and wheat showed that lignin content in the root was higher than in the shoot, and that these levels varied depending on genotype. In wheat, lignin concentration was shown to decrease in seedlings and roots when exposed to mineral deficiencies and increase in response to toxic minerals. Given these findings, our preliminary results, there is a need to further investigate the role of lignin in roots. The overall goal of the project is to determine the role of lignin in wheat roots for drought tolerance and disease resistance and to develop a high-throughput method for lignin analysis in wheat roots and straw. We have worked on processing stem and root tissues for overall lignin content using two independent assays as well as sending pulverized stem and root tissues for analysis of aromatics including lignin monomers to the Ralph lab at University of Wisconsin-Madison. We had good success with lignin extraction in stems, but are still working on lignin extraction from root tissues. As such, we are outsourcing the lignin chemistry to a collaborator who works with grasses and lignin in roots and has already developed protocols. We attempted to implement drought studies using the Phenospex drought spotter in the wheat greenhouse, but had technical issues and then had to abandon work due to the COVID-19 pandemic. Therefore, we focused efforts on molecular and *in silico* approaches to understanding two key enzymes that regulate lignin biosynthesis: CAD and COMT.

Impact

In addition to stress tolerance, lignin has important implications for the rhizosphere and agricultural soils, particularly since it is a stable component of soil organic matter (SOM). There is evidence that lignin slows down the mineralization of nutrients from crop residues. For example, the ratio of lignin to nitrogen is used as an indicator for litter degradation. Studies have shown that lignin negatively affects short-term nitrogen release from different types of green manures that differ in lignin content and that time is a key factor in the lignin/nitrogen equation. Since SOM contains roughly two-thirds of global terrestrial carbon storage and lignin is an important component of SOM, lignified biomass represents a promising source of sustainable fertilizer, which is a concern for Washington state farmers and globally. Our research has shown the lignin monomer content and not total lignin content in winter wheat stems is important for

residue breakdown and thus management. Long-term our data will shed light on the role of lignin in rhizosphere processes as well—such as soil-borne pathogen management and improving overall plant responses to abiotic stresses like drought, salinity, changes in pH, and cold.

Outcomes and Deliverables

Overall, we were able to confirm initial findings that Louise and AUS28451 roots differed in lignin content. We performed some initial experiments examining *Fusarium* resistance in the backcross Lou/Au populations and attempted to relate that to the total lignin content of the lines. At this point, it is unclear whether the lignin content, monomer ratios or another aromatic compound is contributing to the stress tolerance of AUS28451. To address this, we established a collaboration with Dr. John Ralph's lab at the University of Wisconsin-Madison. He is an expert in lignin biochemistry and is working on running a complete aromatic profile panel on Louise and AUS28451 shoots and roots. While we are awaiting the results, we are eager to determine the differences and hope that these analyses shed light on candidate molecules and breeding targets. We did streamline a total lignin quantification and protocol. Our greenhouse work was stymied in year 3 by the COVID-19 pandemic and therefore will continue into 2021. In year 3, we shifted focus to try to understand the genes that control lignin production in wheat to better understand lignin accumulation in the shoots and roots, particularly in response to biotic and abiotic stress. Below we detail some of our findings from the last three years.

Lignin quantification protocol

To establish a rapid method to quantify total lignin, wheat stem samples taken at the second internode after grain filling were ground and sequentially washed using a modified in-house cell wall washing protocol adapted from (Fukushima and Hatfield, 2001). The protocol involves washing uniformly ground plant tissue twice with potassium phosphate, three times with Triton X-100, twice with sodium chloride, twice with distilled water, and three times with acetone. Washed samples are then dried in a 60°C oven for 24 hours and weighed again. The acetyl bromide digestion solution includes NaOH, freshly prepared hydroxylamine-HCL, acetic acid, and acetyl bromide. Samples are digested at 70°C for 30 minutes and then spun down. The suspended solution is then tested on a spectrophotometer at 280nm for absorbance compared to an empty digestion solution control.

Ideally, standards should be generated from commercially extracted lignin derived from monocot sources. Instead, standards were generated from commercially extracted lignin derived from a dicot source due to the lack of a commercially available monocot lignin extract. Exactly 50mg of lignin extract was suspended in 10ml of acetyl bromide digestion solution to generate a working stock solution (stored in a sealable glass container). Seven diluted standards were generated, including a blank sample representing the minimum 0ug/ml of lignin, with a dilution series up to 100ug/ml. All standards were then measured at 280nm on an Eppendorf Biophotometer, and plotted on a graph where measured absorbance at 280nm can be equated to a known lignin concentration (Figure 1).

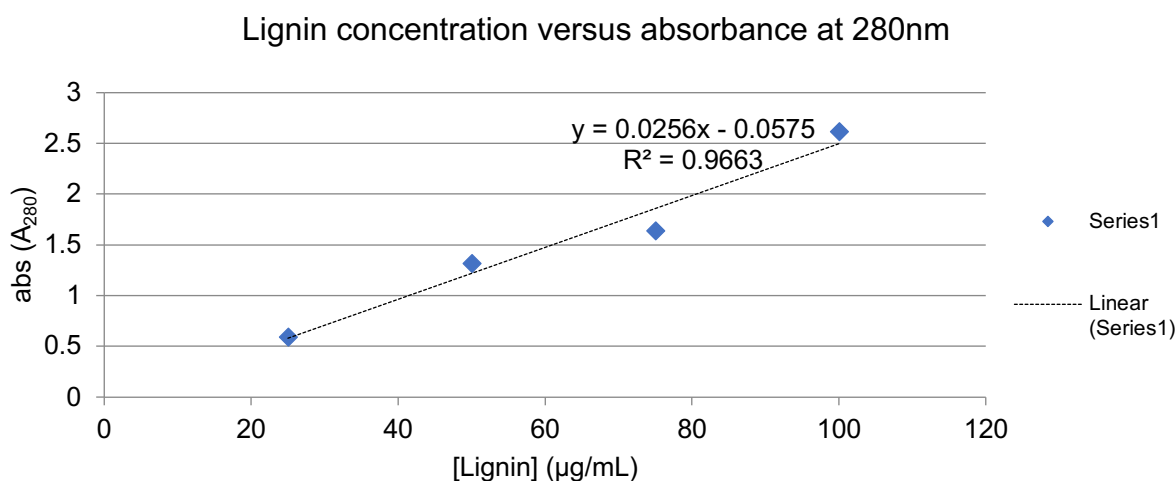


Figure 1: A four-point test standard plot derived from absorbance values of lignin solutions resulting from the acetyl bromide digestion protocol. Measuring light absorption relative to known concentrations of lignin generates a regression curve that can be used to estimate the unknown concentrations of test samples.

This protocol has been used to confirm and test lignin concentrations in the Louise and AUS28451 backcross populations but will be repeated to confirm the initial findings.

Identification of lignin biosynthesis enzymes: CAD and COMT

Lignin is a multifunctional, complex biopolymer that is of critical importance to the overall fitness of terrestrial plants. Evolutionary pressure led aquatic plants to biosynthesize and integrate lignin into their secondary cell walls (SCW), which facilitated the emergence of terrestrial plants that now account for roughly 30% of the total carbon biomass found in the Earth's biosphere. Lignin acts as a mechanical scaffold that enables plants to resist the mechanical forces of gravity and wind. Furthermore, lignification of plant xylary tissue enables plants to resist embolisms caused by the high pressure of upward water transport driven by photosynthesis and evapotranspiration while also providing a hydrophobic surface within xylary tissue that maximizes water retention. Increased lignin deposition has been correlated with drought and salt tolerance. Lastly, evidence suggests lignified tissue provides a protective barrier from both herbivory and pathogen attack.

Lignin itself is primarily composed of three aromatic monomers: p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S). Moreover, there is evidence of additional

monomers that exist but at lower concentrations. Lignin monomer composition is variable and can differ based on species, developmental age, and environment. Lignin biosynthesis begins in the plastids where phosphoenolpyruvate is converted into phenylalanine in the Shikimate pathway. Phenylalanine is shunted into the cytosol, where phenylalanine ammonia-lyase (PAL) commits phenylalanine to the phenylpropanoid synthesis pathway via deamination, generating cinnamic acid. Tyrosine is also deaminated by tyrosine ammonia-lyase (TAL) generating p-coumaric acid, suggesting a redundant substrate intake to the phenylpropanoid pathway. Several cytosolic reactions follow, including the activation of two critical lignin monomer synthesis enzymes: cinnamyl alcohol dehydrogenase (CAD) and caffeic O-methyl transferase (COMT). CAD catalyzes the final step in the biosynthesis of three of the most abundant lignin monomers (S, G, H), which actively converts a phenolic aldehyde into an alcohol group through zinc-mediated hydrolysis of NADPH or NADH. In general, the CAD enzyme belongs to the medium chain dehydrogenase reductase (MDR) family, which is defined by three highly conserved motifs: a structural zinc binding motif, a catalytic zinc-binding motif, and a NADPH/NADH catalytic domain called a Rossmann Fold. COMT has a wide range of substrate affinities. Depending on the organism, COMT participates in multiple steps of phenylpropanoid biosynthesis due to its highly conserved S-adenosyl methionine (SAM) binding domain that acts as a methyl donor in hydroxyl-specific methylation reactions. COMT has been shown to have an affinity for both caffeic acid and hydroxyferulic acid, which becomes ferulic acid and sinapylaldehyde respectively after the fifth hydroxylated position of each ligand is methylated. Moreover, COMT methylates precursors of both coniferyl alcohol and sinapyl alcohol lignin monomers.

The resulting lignin monomers are then quickly transported out of the cell by ATP-binding cassette (ABC) transporters into the apoplast during construction of the SCW. There, lignin monomers are oxidized by laccases and peroxidases, creating lignin radicals that proceed to polymerize via carbon-carbon and ester bonds. Though the polymerization of lignin monomers is highly complex, evidence suggests that the polymer organization is not random, but the mechanisms controlling polymerization have yet to be defined. Both the phenolic chemical basis of the lignin monomers and the carbon-carbon and ester bonds that join the phenolic bases result in tissue that is recalcitrant to degradation. Hydrolysis of the carbon-carbon bonds and ester bonds is energy intensive and generates phenolic free radicals that prove to be highly toxic to the herbivore. Such toxicity and recalcitrant digestibility acts as an innate defense from pathogen attack, and has been observed in several plant species including *Oryza sativa* L., *Populus trichocarpa*, *Arabidopsis thaliana*, *Nicotiana tabacum*, *T. aestivum*, and *Zea mays* L. However, fungal organisms such as *D. citrispora* (white rot) and *P. placenta* (brown rot) have evolved strategies to digest lignified tissue by either reducing the phenolic radicals formed by digestion (white rot), or by digesting the vast quantities of cellulose and hemi-cellulose that surround the lignin polymer (brown rot).

An exhaustive list of all TaCAD protein sequences was generated by BLASTp search of the EnsemblPlants database (version 42 accessed April 2019)

<https://plants.ensembl.org/index.html>], using the protein sequence of the *bona fide* BdCAD1 (Bradi3g06480). The gDNA, cDNA, protein sequences, all physical properties, and location and orientations of 47 unique putative *TaCAD* candidates were organized, given unique names, rooted in the order by which they appear in the *T. aestivum* genome, phylogenetic class, and chromosomal location. CAD enzymes fall into three classes with Class I being the *bona fide* CAD also known as CAD1 in other plant species.

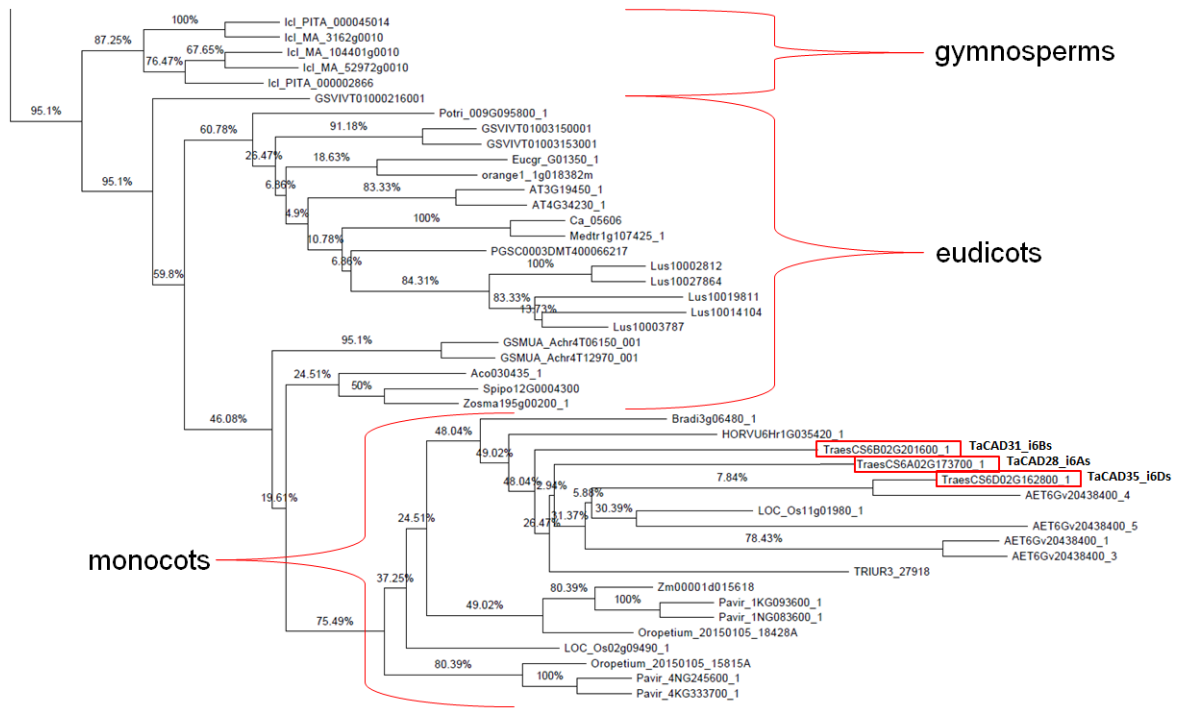


Figure 2: Class I CAD subtree. Sequences from gymnosperms, eudicots and monocots segregated into their own subclasses. *T. aestivum* CADs are boxed in red and given common names for simplicity.

The intronic and exonic boundaries of all 47 *TaCAD* genes were resolved by utilizing the Gene Structure Display Server [<http://gsds.cbi.pku.edu.cn/>]. All *TaCAD* protein sequences were aligned relative to *bona fide* CAD sequences *AtCAD4* and *AtCAD5*, *OsCAD2*, *BdCAD1*, and *TaCAD1* using the MUSCLE algorithm provided in the Mega X software suite, where the MDR motifs were identified. Of particular note, *TaCAD1* (Genbank GU563724) corresponds to the EnsemblPlants gene ID TraesCS6D02G162800 which was renamed *TaCAD35_i6Ds(TaCAD1)*. All *TaCAD* protein sequences were then subjected to a phylogenetic analysis to define what classification each putative *TaCAD* falls under and Class I CADs or the *bona fide* CAD proteins are displayed (**Figure 2**). The phylogenetic tree is rooted with CAD sequences from *Aegilops tauschii* (and CAD paralogs), *Anasus comosus*, *Arabidopsis thaliana*, *Asparagus officinalis*, *Brachypodium distachyon*, *Cicer arietinum*, *Hordeum vulgare*, *Linum usitatissimum*, *Musa acuminata*, *Medicago sativa*, *Nicotiana benthamiana*, *Oropetium thomaeum*, *Oryza sativa*, *Panicum virgatum*, *Physcomitrella patens*, *Spirodela polyrhiza*, *Triticum urartu* (and CAD paralogs), *Zea mays*, and *Zostera marina*.

To determine the cues and signaling pathways that govern their expression, a promoter analysis of all *TaCAD* genes was performed. The 5' UTR and 2000bp upstream of the 5' UTR was queried in the PlantCARE promoter element database. The resulting data was collected and compiled into a graph that delineates the number of cis-regulatory elements found in that particular region. We found cis-regulatory elements that are involved in drought responses,

wounding and pathogen response, heat, and light (Figure 3). These data indicate further study of these genes in wheat is warranted.

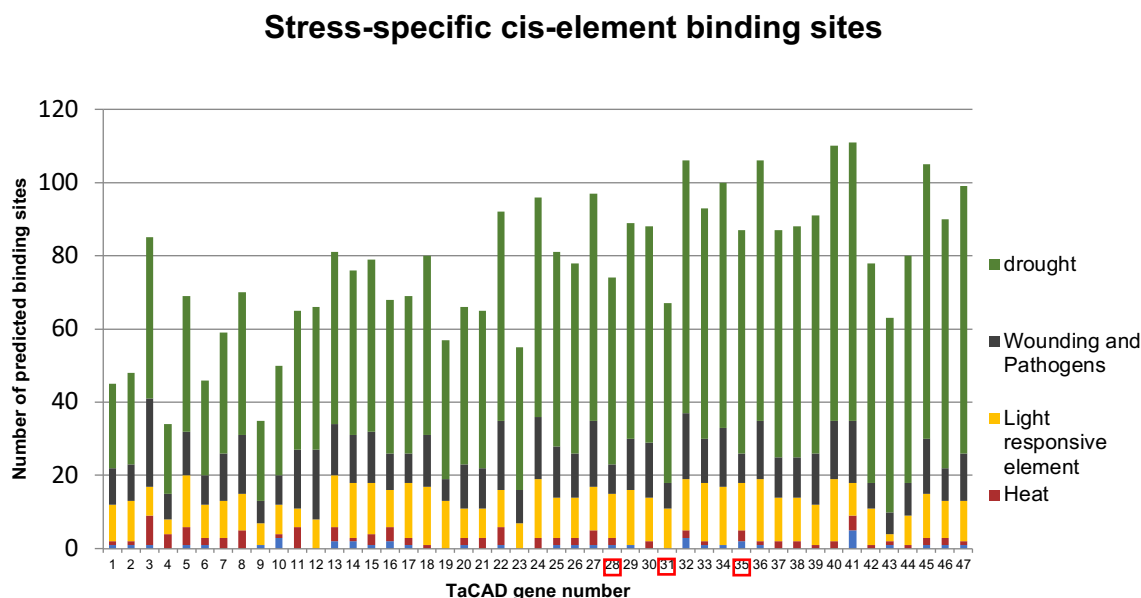


Figure 3: Number of stress-related transcription factor (TF) binding sites found in TaCAD1-47 cis-regulatory elements. The class I *TaCADs* *TaCAD28_i6As* and *TaCAD35_i6Ds* (*TaCAD1*) (highlighted in red) contain cis-regulatory elements for drought, wounding and pathogens, light, heat, and cold. *TaCAD31_i6Bs* promoter contains TF binding sites for drought, wounding and pathogens, and light, but lacks cis-regulatory elements responsive to heat and cold. All *TaCADs* appear to have cis-regulatory elements responsive to drought, wounding and pathogens, and light exposure.

Recently, TaCOMT-3DL has been found to contribute to resistance of sharp eyespot disease and overall stem mechanical strength. Moreover, the *COMT* gene family in *Z. mays*, *O. sativa*, *B. distachyon* and *Sorghum bicolor* have received attention from research groups interested in reducing stem lignin concentration to increase cellulose bioavailability for biofuel production and livestock forage. We performed preliminary analysis of the recently published *T. aestivum* cv. Chinese Spring genome and found 50 TaCOMT isoforms with a protein sequence similarity of >40% to BdCOMT4 (Bradi3g16530) and an additional 4 TaCOMT paralogues (TaCOMTp) containing the SAM domain. Given the volume of recently acquired preliminary data and the newly available sequenced hexaploid wheat genome of Chinese Spring, it is an opportune time to recharacterize both the *TaCAD* and *TaCOMT* gene families in their entirety. Analysis of TaCAD and TaCOMT will add value to the analysis because such context will give breeders an objective molecular basis to pursue new and potentially improved breeding targets.

Conclusions

While our progress was hindered by the pandemic and challenges with supply chain issues and ability to physically be in the laboratory, we tried to make the best of the situation and refocused efforts on the molecular nature of lignin biosynthesis. The focus of our next proposal will be to resolve the relationships between lignin, root architecture and biotic and abiotic stress to help breeders select for more resilient root systems.

WGC project number: 13C-3019-3687
WGC project title: A Genetic Arsenal for Drought Tolerance, Getting to the Root of the Problem
Project PI(s): Karen A. Sanguinet, Kim Garland-Campbell, Timothy Paulitz
Project initiation date: 7/1/18
Project year (X of 3-yr cycle): year 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
1. Quantification of lignin content in roots and stems	A robust and reliable method to accurately determine lignin content in root samples in a high-throughput manner is the main deliverable of this objective.	The first rounds of lignin extractions were performed with the parental lines: AUS28451 and Louise in 2018 both for total lignin and for lignin monomers. Following optimization of the protocols for root extractions, the Lou/Au BC1F6 populations were analyzed in 2019, but another repetition is needed. We have also identified some additional backcross lines of interest. There were technical issues with quantification of the full aromatic profile so we are now outsourcing that to a collaborator.	The quantification of lignin and optimization of the extraction methods was performed for total lignin. Three independent methods have been used for lignin quantification. We are now performing an entire aromatic profile for AUS28451 and Louise. Due to complications and delays from the COVID pandemic, the samples are to be analyzed by Dr. John Ralph's lab in January 2021.	We developed a robust and high-throughput method for lignin quantification, and are planning a methods protocol for publication in addition to another peer-reviewed publication with the addition of the aromatic profiles generated by the Ralph lab.
2. Assessment of the role of lignin in drought	If lignin in roots is associated with drought, this can then be a desirable trait and selected/screened for in breeding populations.	The parental lines were assessed and protocols established for the greenhouse trials on the BC lines and is being assessed in year 3.	Assessment of the parental lines was completed in 2018 and is being followed by two rounds of greenhouse trials with the backcross lines in year 3 that were delayed until Spring 2021.	The findings and protocols will be reported at field days and in peer-reviewed publications. An abstract was submitted for Lind Field Day in June 2020.
3. Root lignin and soil-borne disease	If lignin in roots is associated with disease resistance as hypothesized, this can then be selected/screened for in breeding populations.	The parental lines have been assessed and protocols established for the greenhouse trial in years 1 and 2 and one round of assessment was performed on the BC population, but that must be repeated.	Assessment of disease resistance was assayed in spring 2019, but needs to be repeated in 2020, but was delayed due to COVID and working remotely for most of the team.	The findings and protocols will be reported at field days and in peer-reviewed publications, in Wheat Life magazine, and via WheatBeat podcasts.

**Washington Grain Commission
Wheat and Barley Research Annual Progress Report**

Project #:3144

Progress Report Year: _2_ of _3

Title: ***Breeding Wheat Varieties With Efficient Control of ROS Production***

Researcher: *Andrei Smertenko, Institute of Biological Chemistry, CAHNRS, WSU*

Cooperators: *Dr. Michael Pumphrey, Department of Crop and Soil Sciences.*

Executive summary: *This project aims to advance toolbox for breeding drought and heat tolerant wheat varieties. Our approach is based on the fact that harsh environmental conditions, including heat and drought, increase production of free radicals also known as Reactive Oxygen Species (ROS). ROS diminish the yield by damaging cells inside plant body. Plants alleviate the ROS damages using so-called "scavenging" mechanisms. Varieties with higher scavenging activity would yield better in hot and dry climates. We want to identify genetic markers with more efficient ROS scavenging and introduce these markers into breeding programs. Previously, our laboratory developed a technique for measuring ROS scavenging under the greenhouse condition. In Year 1 (growth season 2019) the suitability of our technique for analysis of material in the field was tested using 14 spring wheat varieties in Lind, Moses Lake, and Spillman farms. We found that our technique detected variability of the ROS scavenging in the field-grown material. One of the tested varieties, Kelse, was amongst varieties with more efficient ROS scavenging. In Year 2 we collaborated with Dr Pumphrey to phenotype ROS scavenging in 180 RILs of the biparental population Kelse x Scarlet in Lind. This RIL population has been genotyped. It means we can use this material to identify genetic markers of efficient ROS scavenging. The material was collected on May 29 when the maximum day temperature reached 88°F. Despite delays caused by COVID-19 pandemics, we completed the measurements. The results demonstrate significant variability of ROS scavenging activity in the population.*

Impact: *We produced phenotyping data on ROS scavenging in the Kelse x Scarlet population under heat and drought stress. This information will be used to identify genetic marker of efficient ROS scavenging. In the long-term this will contribute to breeding drought and heat-tolerant wheat varieties. The project provided training to undergraduate student Jessica Fisher, graduate student Kathleen Hickey, and post-doctoral scientist Taras Nazarov.*

WGC project number:	3144
WGC project title:	Breeding Wheat Varieties With Efficient Control of ROS Production
Project PI(s):	Smertenko
Project initiation date:	June 10, 2019
Project year (X of 3-yr cycle):	2 of 3

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**Washington Grain Commission
Wheat and Barley Research Annual Progress Report**

Project #: 3690

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

Title: Developing Washington Wheat with Stable Falling Numbers (FN) through Resistance to Preharvest Sprouting and LMA.

PIs: Camille M. Steber, Michael O. Pumphrey, Arron H. Carter, and Kimberly Garland Campbell

Cooperators: Deven See, Craig Morris, Aaron Esser, and Drew Lyon

Executive summary: The goal is to breed for stable Falling Numbers (FN) in Washington wheat through selection for genetic resistance to preharvest sprouting and late maturity alpha-amylase (LMA), the two main causes of low FN. The project identified cultivars with low FN problems through FN testing of the WSU cereal variety trials. Preharvest sprouting and LMA testing results were provided to the WSU and USDA breeding programs to enable selection from improved FN. Genes or loci associated with preharvest sprouting and LMA tolerance were identified by mapping using greenhouse and field-testing results. We have identified molecular markers linked to LMA and PHS resistance to allow selection in early breeding lines. We improved LMA testing methods. This is the final report for the FN project. A new proposal was not submitted. However, Dr. Steber would like to continue to present research plans and achievements during the annual Wheat Research Review.

Objective 1. Identify varieties with stable FN by performing FN tests and statistical analysis of variety trials in environments that have preharvest sprouting and/or LMA.

FN data from the soft white winter WSU variety trials was analyzed using five statistical methods designed to examine the impact of genetics and the environment. FN is difficult to analyze because it is impacted by multiple environmental factors leading to preharvest sprouting or LMA. The factor analytic model provided the best fit for this complex dataset and will be used to compare variety performance for falling number and how stable that falling number is in challenging environments.

FN testing was performed in all three years of the project (data posted on <http://steberlab.org/project7599.php>). There were only mild problems with low FN in 2020. If we look at soft white winter wheat, in 2020 only 5 of the 18 locations had mild to moderate problems with low FN whereas in 2019, 15 of 20 locations had FN problems and four of these locations had severe problems. In a preliminary survey of susceptible checks in 2020, only 3 hard winter, 4 soft white spring, and 2 hard spring locations had low FN.

Objective 2. Screen winter and spring wheat breeding lines for preharvest sprouting and/or LMA. PHS and LMA testing were performed both on elite breeding lines and on mapping populations in order to improve selection for higher FN varieties and identify important loci associated with these traits. In 2018, 1335 lines were screened for LMA and 708 for preharvest sprouting susceptibility by spike-wetting test. In 2019, 1113 samples were screened for LMA, and 810 were screened for PHS susceptibility by spike-wetting test. In 2020, 1440 samples were tested for LMA and 3,229 for PHS.

Objective 3. Identify molecular markers linked to LMA susceptibility in northwest wheat.

a. We completed a genome-wide association study of the hard red spring TCAP population (250 lines) and identified six putative genes/loci associated with LMA phenotype. Of these 6,

QLMA.wsu.3B , *QLMA.wsu.6B* , and *QLMA.wsu.7B* were located close to LMA QTLs previously mapped in Australian or CIMMYT wheat lines (Mrva and Mares, 2001; Tan et al., 2004; McNeil et al., 2009; Emebiri et al., 2010). We will examine if these markers predict LMA in PNW breeding lines by examining their association with LMA in the WSU Cereal Variety Trials (collaboration with Deven See). b. Two winter RIL populations, Cara x Xerpha and Xerpha x Bobtail, were screened for LMA in 2019 and 2020. A genetic map was developed by K. Garland Campbell for the Cara/Xerpha population. This will allow us to perform a preliminary QTL analysis in 2021. A genetic map will need to be developed for the Xerpha/Bobtail population obtained from R. Zemetra in collaboration with Deven See.

Table 1. Putative significant QTL from GWAS for LMA.

¹ GH QTL	Marker	Chr	Position	-log ₁₀ (p)	² Effect	MAF	³ Fav allele	n
<i>QLMA.wsu.3B</i>	IWB63008	3B	801300	8.6	0.08	0.10	A/G	242
<i>QLMA.wsu.1A</i>	IWB35476	1A	1512200	6.4	-0.05	0.33	T/C	120
<i>QLMA.wsu.6B</i>	IWB26762	6B	1104500	7.0	-0.07	0.11	T/C	120
Field QTL								
<i>QLMA.wsu.3A</i>	IWB11852	3A	207400	6.9	0.16	0.26	A/C	206
<i>QLMA.wsu.7B</i>	IWB25774	7B	1335900	9.8	0.16	0.25	A/G	206
<i>QLMA.wsu.7D</i>	IWB48862	7D	861900	9.9	0.17	0.24	T/C	206

¹GH = greenhouse

²The positive Effect values indicate elevated risk of LMA phenotype in N. America hard red spring wheat TCAP association panel

³The major allele that reduce the susceptibility of LMA in N. America hard red spring wheat TCAP association panel

Objective 4. Develop molecular markers for selection of PHS resistance in northwest wheat. Genome-wide association mapping was conducted in two related populations based on spike-wetting tests and falling numbers after artificial or natural rain (see Martinez et al., 2018). Some loci linked to good seedling emergence did not correspond to loci for preharvest sprouting resistance, suggesting that we may be able to select preharvest sprouting resistant without compromising seedling emergence. A preliminary genomic selection model was developed in the hope of selecting PHS tolerance without compromising emergence.

Impact: Wheat in all market classes is dramatically discounted for low falling numbers (below 300 sec). Moreover, a consistent problem with low FN could damage the reputation of Washington wheat in foreign markets. Screening for increased genetic resistance to low FN, LMA, and preharvest sprouting will enable the selection of new varieties with more stable FN. Currently, screening breeding lines for PHS and LMA is a labor-intensive process that is only feasible for mid to late breeding lines. The identification of molecular markers associated from preharvest sprouting and LMA tolerance will allow selection for improved genetic resistance in earlier breeding material and should greatly improve the FN of new varieties. Posting of FN data on the WSU small grains website and the PNW FN website (<http://steberlab.org/project7599.php>) makes this data available to farmers and to breeders.

Publications:

1. Steber, C. M., Carter, A. H., & Pumphrey, M. O. (2018, January). Hunting for genes: falling numbers project seeks to reduce risk by breeding for genetic resistance. *Wheat Life*, 61(1), 50–51.
2. Martinez, S. A., Godoy, J., Huang, M., Zhang, Z., Carter, A. H., Garland Campbell, K. A., & Steber, C. M. (2018). Genome-Wide Association Mapping for Tolerance to Preharvest Sprouting and Low Falling Numbers in Wheat. *Frontiers in Plant Science*, 9, 1–16, <https://doi.org/10.3389/fpls.2018.00141>.
3. Delwiche, S. R., Higginbotham, R. W., & Steber, C. M. (2018). Falling number of soft white wheat by near-infrared spectroscopy: A challenge revisited. *Cereal Chemistry*, 95(3), 469–477.
4. Steber, C. M., & Garland Campbell, K. A. (2019, April). Rising optimism fuels falling numbers research. *Wheat Life*, 62(4), 40–52.
5. Sjoberg, S., Carter, A. H., Steber, C. M., & Garland-Campbell, K. A. (2019). Unravelling Complex Traits in Wheat: Approaches for Analyzing Genotype \times Environment Interactions in a Multi-environment Study of Falling Numbers. *Crop Science*, 60, 3013–3026.
6. Martinez, S. A., Shorinola, O., Conselman, S., See, D., Skinner, D. Z., Uauy, C., & Steber, C. M. (2020). Exome sequencing of bulked segregants identified a novel TaMKK3-A allele linked to the wheat ERA8 ABA-hypersensitive germination phenotype. *Theoretical and Applied Genetics*, 133, 719–736.
7. Sjoberg, S. M., Carter, A. H., Steber, C. M., & Garland-Campbell, K. A. (2020) Application of the factor analytic model to assess wheat falling number performance and stability in multi-environment trials. *Crop Science*, doi:10.1002/csc2.20293.
8. Liu, Chang, KM Tuttle, KA Garland Campbell, M O. Pumphrey, C.M. Steber. Investigating Conditions That Induce LMA Using Northwestern U.S. Spring Wheat (*Triticum aestivum* L.). *Seed Science Research*. Submitted and accepted with minor revision.
9. Liu, Chang, RS. Parveen, SR. Revolinski, KA Garland Campbell, M O. Pumphrey, C.M. Steber. The Genetics of LMA in North American Spring Wheat (*Triticum aestivum* L.). *Seed Science Research*. Submitted and accepted with minor revision.

WGC project number: 3690
WGC project title: Developing Washington Wheat with Stable Falling Numbers (FN) through resistance to preharvest sprouting and LMA
Project PI(s): Camille M. Steber, Michael O. Pumphrey, Arron H. Carter, and Kimberly Garland Campbell
Project initiation date: July 1, 2018
Project year (X of 3-yr cycle): This is year 3 of 3 of the funding cycle.

Objective	Deliverable	Progress	Timeline	Communication
1. Identify varieties with stable FN by performing FN tests and statistical analysis of variety trials in environments that have PHS and/or LMA.	1. FN testing results posted on the PNW FN website (stebberlab.org). 2. Development of statistical methods to compare varieties for performance and stability of FN. 3. A new FN comparison tool.	1. FN testing of spring and winter varieties has been completed for all variety trial locations that showed a low FN problem in 2017, 2018, and 2019. 2. The 2020 field season did not have wide-spread problems with low FN. FN testing is underway. 3. Two articles by Sjöberg et al were published in Crop Science exploring statistical methods to analyze FN data based on the WSU soft white winter variety testing in 2013, 2014, and 2016. The factor-analytic model in AMSREML provided the best tool for comparing varieties based on overall FN performance and stability under pressure in challenging environments. 4. We discovered that as an FN machine ages, it can give highly inaccurate FN measurements in the FN 250-350 sec range. 5. An FN comparison tool has been added to the small grains variety selection tool based on FN in 2019. This tool will be improved to include data over the years analyzed using a factor analytic model. This will require enhanced curation and analysis of the data.	1. Annually, FN testing of susceptible varieties will be used to determine which WSU Cereal Variety locations will be subject to FN testing. 2. In 2018 and 2019, compare various methods for ranking varieties for FN. 3. In 2019, complete statistical analysis of soft white winter FN data from 2013, 2014, and 2016. 4. In 2020, select a method for annual analysis of variety trial FN data.	Results of annual FN testing have been made available on the PNW FN website (stebberlab.org) and on the WSU small grains website. Information has been published in peer-reviewed journals, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review.
2. Screen winter and spring wheat breeding lines for PHS and LMA susceptibility.	Data obtained will allow selection for increased resistance to LMA and preharvest sprouting in winter and spring wheat breeding programs at WSU. This should indirectly lead to release of varieties with increased resistance to low FN.	1. LMA field-testing was performed in 2018, 2019, and 2020, screening about 1400 lines per year including winter and spring wheat breeding lines, variety trial and mapping populations. Enzyme assays of 2020 material are still in progress. 2. Preharvest sprouting tolerance was tested using greenhouse spike-wetting tests of spikes harvested at physiological maturity from the field. The PHS testing was expanded in 2020 to include 1,683 plots of breeding lines and 1,534 mapping lines. 3. Experiments were performed to optimize the conditions for LMA induction. 4. In 2020, doubled haploid populations were screened both for fall emergence and for preharvest sprouting tolerance at maturity. This will be used to generate a genomic selection model.	1. Perform field LMA testing annually of about 1000 lines annually for breeding and mapping. 2. Perform spike-wetting tests annually. 3. Continue improving methods to increase efficiency.	Information has been published in peer-reviewed journals, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review.
3. Identify molecular markers linked to LMA susceptibility in northwest wheat.	1. Molecular markers linked to LMA resistance allowing selection in earlier generation breeding lines. 2. Mapped LMA genes/loci linked to LMA resistance and susceptibility in the soft white spring TCAP population. 3. Mapped LMA genes/loci linked to LMA resistance and susceptibility in recombinant inbred line populations.	1. Six loci/genes associated with LMA in spring wheat were identified in 2020 on chromosomes 1A, 3A, 3B, 6B, 7A and 7B. The loci on 3B, 6B, and 7B are close to LMA loci previously mapped by Australian researchers. Ongoing research will determine if these 6 molecular markers or nearby markers can select for LMA resistance in PNW breeding lines. 2. In another project, the parents for 10 spring RIL populations and 20 winter RIL populations were screened for LMA. Two promising winter 2-parent populations were chosen for LMA mapping, Cara x Xerpha, and Xerpha x Bobtail. 3. LMA field testing was performed on Cara/Xerpha and Xerpha/Bobtail in 2019 and 2020. A preliminary QTL analysis of Cara/Xerpha will be conducted in 2021. 4. LMA testing results revealed that tall wheat (<i>rht</i> wild-type) tend to induce LMA without a cold shock treatment. Because this LMA phenotype was much more consistent than any we've seen, future mapping efforts will focus on tallxtall crosses.	1. The goal to complete TCAP LMA screening in 2018 must be extended into 2019. 2. Genome-wide association mapping in the TCAP was to be completed in 2019. 3. RIL populations for LMA mapping will be identified by 2019, and LMA screening initiated in 2019 and 2020.	The Association Mapping study has been provisionally accepted for publication in the journal Seed Science Research. Information has been summarized in Wheat Life articles, in presentations at the Wheat Quality Council, Western and Eastern wheat workers meetings, presented during field days, and presented at the annual Wheat Review.

4. Develop molecular markers for selection of preharvest sprouting resistance in northwest wheat.	1. Molecular markers that can be used to select for resistance to preharvest sprouting. 2. Identify markers that can select for sprouting resistance without compromising field emergence.	1. Mapping results for preharvest sprouting were based on FN and sprouting scores from spike-wetting tests. Mapping was also performed for emergence based on field emergence and coleoptile/seedling elongation. Comparison found that there were some strong genes/loci linked to emergence that were not linked to preharvest sprouting susceptibility. This is a preliminary result, but suggests that this mapping approach may be used successfully in soft white winter wheat populations. 2. A preliminary genomic selection model was developed in 2020. 3. In 2021, spike-wetting tests and field emergence scoring were performed for 800 doubled-haploid winter wheat breeding lines derived from parents in the QAM and SNP winter wheat mapping populations. These data will be used to confirm marker-trait associations for molecular markers.	1. In 2018, GWAS was performed in a second population to confirm marker-trait associations. 2. In 2018, spike-wetting tests were performed on winter doubled haploid populations. 3. In 2019 and 2020, we will develop a genomic prediction model.	Information has been published in the peer-reviewed journal TAG, summarized in Wheat Life articles, presented in the FN workshop at the PNW-Wheat Quality Council, during field days, and at the annual Wheat Review. The goal is to summarize these markers on the PNW falling number website to make it easier for wheat breeders to access this information.

Project #: 126593

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

Title: Intelligent Prediction and Association Tool to Facilitate Wheat Breeding

PI: Dr. Zhiwu Zhang

Cooperators: Dr. Michael Pumphrey, Dr. Arron H. Carter, and Dr. Kimberly Campbell

Executive summary: *We updated one software package, released a new software package, and published one article partially under support of this project in this fiscal year. The GAPIT software package updated for new functions for both GWAS (Genome Wide Association Study) and GS (Genomic Selection) (<http://zzlab.net/GAPIT>). GAPIT is R Package for users with programming skills in R language. Analyses can be programmed to process large amount of analyses with same settings. The new software package is MMAP (<http://zzlab.net/MMAP>). The manuscript of update GAPIT (version 3) is under review by Genomics, Proteomics and Bioinformatics. The manuscript is released to public b [preprint](#). The MMAP was published by [Bioinformatics](#).*

Impact: *Our collaborative research positions WSU/USDA-ARS research team as one of the the world's leading institutions to conduct fundamental and applied research, publish academic articles, and update and release software packages. Our project's success not only benefit Washington, but will also benefit the entire world through the dissemination of knowledge. In short term, breeders can conduct most of data analyses without frustration on data formatting and selecting different analytical functions. They have more oppertunities to find the causative genes controlling traits of interest. They have more confidence to eliminate lines with low genetic potentials to reduce the cost of field trials. In long term, breeders have more chances to retain the genetic lines with desirable genes, and recombine them to create superior varieties.*

WGC project number: 126593
WGC Project title: **Intelligent Prediction and Association Tool to Facilitate Wheat Breeding**
Project PI(s): *Zhiwu Zhang, Michael Pumphrey, Arron H. Carter, and Kimberly Campbell*
Project initiation date: 1-Jul-18
Project year: 3 of 3

Objective	Deliverable	Progress	Timeline	Communication
3) Develop a web-driven molecular breeding pipeline to enable breeders to conduct analyses and make decisions anywhere with internet access.	New tools named MMAP with integration of web based data and calculation through internet access.	We have developed iPat under previous WGC support. The software has graphic user interface and ability to use third-party packages as internal engines for data analyses. The functions of iPat will be transformed into web based software.	December 31, 2020: Functions have been developed ; June 30, 2021: Complete system testing on real data from all the Co-PIs.	1) One presentation at WGC annual meeting; 2) One presentation at International Conference of Plant and Animal Genome; and 3) one paper was published by Bioinformatics for MMAP.