Executive summary: In 2023, studies were conducted according to the objectives of the project proposal, and all objectives specified for the second year of this funding cycle were completed on time. 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, resistance, and mechanisms of interactions between the rust pathogens and plants.

Introduction: Wheat is grown in more than 2 million acres in the State of Washington (WA), and barley is an important crop for producing grain for animal feed, malting, and human food. Stripe rust, leaf rust, and stem rust all can damage wheat and barley crops, especially stripe rust that is the most important disease on wheat in the Pacific Northwest (PNW). Severe stripe rust occurs almost every year on wheat and has the potential to cause yield losses of over 90% on susceptible varieties with an average of potential yield loss of 41% in the PNW from 2002 to 2023. Despite most wheat varieties currently grown in the PNW have variable levels of resistance and the overall resistance in commercial varieties significantly reduces the potential grain yield loss from 41% to 7.8% on average. The 7.8% grain is over 100 million dollars for Washington wheat growers. To further reduce the yield losses of commercially grown varieties, growers need to spend multimillion dollars on fungicide application. Therefore, we will continue focusing on stripe rust and include leaf rust and stem rust in our programs. Our goal is to reduce rust damage to minimal using best possible approaches on a yearly basis. Meanwhile, we will conduct research for new knowledge, resources, and methods to improve control of the rust diseases. The specific objectives of this project are to 1) Improve the understanding of rust disease epidemiology and the pathogen populations; 2) Improve rust resistance in wheat and barley varieties; and 3) Improve the integrated management of rust diseases.

Approach: For Objective 1, we conducted field rust surveys and collect rust samples. We tested stripe rust samples on the wheat differentials and barley differentials to identify virulent races of the pathogens, and determine race frequencies, distributions, dynamics, and potential impact of new races on resistant germplasm, commercially grown varieties, and breeding lines; and select a set of races for evaluating germplasm and breeding lines. In addition, we used molecular markers in combination with the virulence data to identify population changes of the stripe rust pathogens. For leaf rust and stem rust, we collected samples from the PNW and send them to the ARS Cereal Disease Laboratory in Minnesota for identification of races and characterization of populations. 2) For Objective 2, we evaluated wheat and barley lines from breeding programs for resistance to rusts and provide data to breeding programs for developing and releasing new varieties with adequate and durable resistance. We have continue identifying and mapping effective and new genes in wheat and barley for resistance to stripe rust using both genome-wide association study (GWAS) and bi-parental populations. We collaborated with other programs in
identifying and utilizing genes for resistance to stem rust and leaf rust. 3) For Objective 3, we tested new chemicals, together with registered fungicides, for their efficacy of controlling stripe rust in the fields for both winter and spring crops. We provided the chemical efficacy data to individual companies for registration of new fungicides. We will test major commercially grown varieties in field experiments under natural or artificial infection of the stripe rust pathogen with and without fungicide application to determine yield loss by stripe rust and yield increase by fungicide application for individual varieties. These data can be used to guide stripe rust management for individual varieties in combination with rust forecasts and field survey data.

Results: 1) In 2023, we made stripe rust forecasts based on the winter weather data in January and March, conducted field surveys and made recommendations for rust management throughout the crop season. Stripe rust was severe in western Washington but occurred at a low level in the east part of the state due to the lasting drought conditions from May to July. 2) We identified 21 wheat stripe rust races and 5 barley stripe rust races from 287 samples from throughout the U.S., including 18 (86%) wheat stripe rust races and 4 (80%) barley stripe rust races identified from 213 (73%) samples collected by ourselves in Washington. 3) We have completed SSR (simple sequence repeat) genotyping of the stripe rust collections up to 2022 and used the data to determine the pathogen genetic changes. We conducted experiments to convert secreted protein (SP) gene-based single nucleotide polymorphism (SNP) markers to more efficient Kompetitive allele-specific PCR (KASP) markers for monitoring pathogen virulence. 4) We evaluated more than 16,000 wheat and barley entries for resistance to stripe rust in multiple locations and in the greenhouse with selected races of the pathogens. 5) We identified a new gene, officially named Yr85, for resistance to stripe rust in a wheat line used to differentiate races of the wheat stripe rust pathogen and in a collaborative study mapped five stable quantitative trait loci (QTL) for all-stage resistance and high-temperature adult-plant resistance in a new wheat germplasm PI 660122 previously developed in our program. 6) We tested 19 fungicide treatments for control of stripe rust on winter and spring wheats and tested 24 winter wheat and 24 spring wheat varieties for yield loss caused by stripe rust and yield increase by fungicide application. 7) In 2023, we published 17 journal articles and 3 meeting abstracts.

Impact: 1) The recommendations made based on the stripe rust forecasts and surveys avoided unnecessary use of fungicides, which saved growers multimillion dollars in 2023. 2) The race information is used for guiding breeding programs to use effective resistance genes and selecting rust races to be used in screening germplasm for developing resistant varieties. 3) Analyses of the molecular marker data together with the virulence data of the stripe rust pathogen populations have allowed us better understanding the pathogen genetic changes and the effects of cropping systems, variety resistance, and environmental factors on the dynamics of population variation. 4) From the germplasm screening, we identified new sources of resistance and resistant breeding lines for breeding programs to release new varieties for growers to grow. Using the germplasm screening data of the recent years, we collaborated with breeders in releasing, pre-releasing, or registration of 10 wheat varieties and 1 barley variety in 2023. The germplasm evaluation data were also used to update the Seed-Buying Guide for growers to choose resistant varieties to grow. 5) The identified stripe rust resistance genes/QTL and developed molecular markers are useful for monitoring races of the wheat stripe rust pathogen and developing new resistant varieties. 6) The data of the fungicide and variety tests are used for registering new fungicides and guiding the integrated control of stripe rust in the coming years. 7) Our publications have strong impact on guiding stripe rust research and management in the PNW and other regions in the U.S. and the world.
### Outputs and Outcomes:

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<td>1. Improve the understanding of rust disease epidemiology and the pathogen populations.</td>
<td>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.</td>
<td>All planned studies for the project in 2023 have been completed on time. There is not any major delay, failure, or problem to this objective. The race identification for the 2022 collection was completed and summarized. The data and summary were sent to growers, collaborators and related scientists in January 2023. In the 2023 crop season, we collected and received 287 stripe rust samples throughout the country, of which 213 samples (73%) were collected by ourselves from Washington (WA). We have completed the race ID work for the 2023 samples and detected 21 races of the wheat stripe rust pathogen and 5 races of the barley stripe rust pathogen, of which 18 (86%) wheat and 4 (80%) barley stripe rust races were detected in WA. The frequency and distribution of each race and virulence factors 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 for use in screening for breeding lines of wheat and barley with stripe rust resistance. We have completed SSR (simple sequence repeat) marker genotyping of the stripe rust collections up to 2022 and used the data to determine genetic diversity, differentiation, and dynamics of the pathogen. The results have improved the understanding of stripe rust epidemiology and spore movement among different regions in the U.S. We conducted experiments to convert secreted protein (SP) gene-based single nucleotide polymorphism (SNP) markers to more efficient Kompetitive allele-specific PCR (KASP) markers for monitoring pathogen virulence and made some progress. The virulence and molecular studies have improved the understanding the epidemiology, biology and genetics of the pathogen, and provided information and resources for more efficiently monitoring and managing stripe rusts on wheat and barley.</td>
<td>The race identification work for the 2022 stripe rust samples was completed, summarized, and distributed. The race identification work for the 2023 samples has also been completed; the data analysis been mostly done, and the results are being summarized; and the data and summary will be distributed in January 2024. Molecular marker work for the population genetic studies has been completed and published up to the 2017 collection, completed for the 2018-2022 collections, and is being conducted for the 2023 collection. Progress has been made for the development of KASP markers for monitoring virulence genes in the rust populations.</td>
<td>The rust survey and race data were communicated to growers and researchers through e-mails, websites, project reports, meeting presentations and publications in scientific journals.</td>
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### 2. Improve rust resistance in wheat and barley varieties.

| 1) Stripe rust reaction data of wheat and barley germplasm and breeding lines. | In 2023, we evaluated more than 16,000 wheat and barley 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 at both Pullman and Mt. Vernon locations, and some of the entries were also tested in Walla Walla, Central Ferry, 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. Excellent stripe rust data were obtained from the Pullman (artificially inoculated) and Mt. Vernon (under natural infection) locations. The disease data of regional nurseries were provided to all breeding and extension programs, while the data of individual programs’ 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 2023, we collaborated with public breeding programs in releasing and registered 10 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 2023, we identified a new gene, officially named Yr85, for resistance to stripe rust in a wheat line used to differentiate races of the wheat stripe rust pathogen and in a collaborative study mapped five stable quantitative trait loci (QTL) for all-stage resistance and high-temperature adult-plant resistance in a new wheat germplasm PI 660122 previously developed in our program. In 2023, we obtained excellent stripe rust phenotypic data of 4 bi-parental mapping populations at both Pullman and Mount Vernon locations to validate resistance loci previously identified through the bulked analysis of 40 crosses and preliminarily mapped several stripe rust resistance loci in a couple of the bi-parental populations. 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. |
| 2) Reactions to other diseases when occur. | All 2023 germplasm tests were completed, and the data were provided to collaborators on time. The 2024 winter wheat nurseries were planted in fields in October, 2023. The 2024 spring wheat and barley nurseries will be planted in March-April, 2024. The greenhouse tests of the 2023 winter nurseries were completed and sent to collaborators. The greenhouse tests of 2023 spring nurseries have been completed and will be distributed soon. The 2024 winter nurseries have been conducted during this winter, and will be completed by May 2024. All experiments of the molecular mapping studies scheduled for 2023 have been completed. Mapping populations of winter wheat were planted in fields in October, 2023 and those of spring wheat will be planted in April, 2024 for stripe rust phenotype data. |
| 3) New resistance genes with their genetic information and molecular markers. | 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 e-mails, websites, Seed-Buying Guide, and 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. |
| 4) New germplasm with improved traits. | New germplasm tests were completed, and the data were provided to collaborators on time. The 2024 winter wheat nurseries were planted in fields in October, 2023. The 2024 spring wheat and barley nurseries will be planted in March-April, 2024. The greenhouse tests of the 2023 winter nurseries were completed and sent to collaborators. The greenhouse tests of 2023 spring nurseries have been completed and will be distributed soon. The 2024 winter nurseries have been conducted during this winter, and will be completed by May 2024. All experiments of the molecular mapping studies scheduled for 2023 have been completed. Mapping populations of winter wheat were planted in fields in October, 2023 and those of spring wheat will be planted in April, 2024 for stripe rust phenotype data. |
| 5) New varieties for production. | 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 e-mails, websites, Seed-Buying Guide, and 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. |

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.
3. Improve the integrated management of rust diseases.

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. 6) Wheat Life articles. The information is used for developing more effective integrated control programs based on individual varieties. Disease updates and recommendations will allow growers to implement appropriate control.

In 2023, stripe rust was forecasted and monitored throughout the crop season, and rust updates and advises were provided on time to growers. The recommendations avoided unnecessary use of fungicides, saving growers multimillion dollars. In 2023, we planted field nurseries at Pullman, WA for evaluating 19 fungicide treatments on winter wheat and spring wheat, and 23 winter and 23 spring wheat varieties, plus a non-treated check in each nursery. In the fungicide test on winter wheat, all 19 fungicide treatments significantly reduced stripe rust severity, increased grain test weight, and increased grain yield compared to the non-treated check. The significant yield responses ranged from 23.5 bu/A (26.2%) to 50.3 bu/A (43.2%) with some of the new formulations providing best protection and highest yield. In the fungicide-winter wheat variety trial, the two applications of Quilt Xcel at 14 fl oz/A reduced overall severity value by 98.1% in the susceptible check plots. The fungicide applications also significantly reduced the severity values of 12 commercial cultivars (UI Magic, LCS Jet, Curiosity CL+, Otto, Mela CL+, Stingray CL+, ARS-Crescent, Piranha, Keldin, WB4303, Pritchett, and LCS Artdeco), and the reduction ranged from 3.3 to 56.3%. The fungicide applications significantly protected grain test weight of the susceptible check by 5.4 lb/bu and three commercial varieties (UI Magic, Curiosity CL+, and Stingray CL+) by 1.3 to 1.6 lb/bu. The fungicide applications made significant yield differences for the susceptible check (32.6 bu/A more in the sprayed plots) and six commercial varieties (UI Magic, LCS Jet, Curiosity CL+, Otto, Mela CL+, and Stingray CL+) with 12.0 to 27.0 bu/A more in the sprayed plots. The remaining 17 commercial varieties (LCS Hulk, ARS-Crescent, Sockeye CL+, Piranga CL+, Kelding, WB4303, SY Assure, Pritchett, LCS Shine, Northwest Tandem, AP Exceed, Northwest Duet, LCS Jefe, LCS Artdeco, Resilience CL+, M-Press, and SY Dayton) showed no significant yield differences between the no-spray and spray treatments, indicating adequate resistance. These data indicated that stripe rust caused yield loss of 32.6 bu/A (32.6%) on the susceptible check and 6.7 bu/A (5.0%) yield loss on average across the commercially grown varieties under the disease pressure created by artificial inoculation in the experimental field. The fungicide test on spring wheat varieties did not show significant differences between no-spray and sprayed plots for any of the tested varieties, indicating that fungicide application is not necessary under the low rust. The significant yield differences among the varieties is useful for selecting high-yielding varieties to grow.

For this objective, all tests scheduled for 2023 were completed. For the 2023-24 growing season, the winter wheat plots of the fungicide and variety yield loss studies were planted in October, 2023, and the spring plots will be planted in April, 2023. The tests will be completed in August (for winter wheat) and September (for spring wheat), 2024.

The 2023 results were communicated to growers and collaborators through e-mails, project reports and reviews, reported in Wheat Life (the 2022 data), and submitted scientific journals.