FOR ADMINISTRATIVE USE
Program Area Code Proposal Code

Minnesota Wheat Research and Promotion Council

RESEARCH PROPOSAL GRANT APPLICATION

1. NAME AND ADDRESS OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE

Name: Regents of the University of Minnesota
Address: Sponsored Projects Administration
454 McNamara Alumni Center, 200 Oak Street SE
Minneapolis, MN 55455-2070

2. TITLE OF PROPOSAL
Research on Bacterial Leaf Streak of Wheat

3. PRINCIPAL INVESTIGATOR(S)
Ruth Dill-Macky

4. PI #1 BUSINESS ADDRESS
Department of Plant Pathology
495 Borlaug Hall, 1991 Buford Circle
University of Minnesota
St. Paul, MN 55108

5. PROPOSED PROJECT DATES (Jan 1 – Dec 31)
January 1, 2022 – December 31, 2022

Note: Annual Research Reports are Due November 15th

6. TOTAL PROJECT COST
$53,000

7. PI #1 PHONE NO.
612-625-2227 (office)
651-399-0947 (cell)

8. RESEARCH OBJECTIVES: (List objectives to be accomplished by research grant)

1. Validate molecular assays as tools to rapidly and reliably identify *Xtu* in plant tissues and soil samples.
2. Determine where in the wheat seed the bacterium is surviving.
3. Determine how long the bacterium is surviving in association with wheat seed.
4. Examine the efficacy of seed treatments in reducing *Xtu* in association with seed.
5. Conduct field trials in collaboration to examine the efficacy of commercial foliar treatments on BLS.

Attach a 2-page detailed discussion of importance of the proposal to wheat profitability; how study complements previous research in area; procedures to be used; and competency of the research group in achieving research objectives. (Please keep the proposal concise, only 2 pages will be provided reviewers).

Signature

Date

5-January-2022

Minnesota Wheat Research and Promotion Council
RESEARCH PROJECT PROPOSAL
(2-pages maximum)

Abstract: This project continues our efforts to address the control of bacterial leaf streak (BLS) of wheat. The ultimate goal of the project is to deliver economic disease control measures to growers. Our proposed work will further examine the biology of the BLS pathogen with the aim of uncovering avenues of disease control that compliment host resistance. Outcomes of practical value to the wheat grower include understanding the role that seed plays in the survival of the pathogen, the validation of tools to identify the bacterium that incites BLS within seed, crop debris and in soil samples, along with the testing of treatments to disinfest seed. Our work in 2021 indicated that infested seed does pose an increased risk to the subsequent crop, and we have tested protocols to quantify bacteria in seed. We propose to adapt this test in 2022 to other matrices (crop residues and soil) and to test a protocol that should differentiate between living and dead bacterial cells. We also plan to examine the efficacy of seed treatments in disinfesting seed that is contaminated with the pathogen. We also plan to evaluate a number of foliar treatments, both chemical and biological, for the control of BLS in the field.

Describe the background for your proposed project and the importance of this project to the profitability of wheat production in MN: Bacterial leaf streak (BLS) of wheat, caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is presently the most important foliar disease of wheat in Minnesota. Managing BLS is difficult, as fungicides are largely ineffective against bacterial pathogens. Previous work, funded by the MWRPC, has enabled us to establish a regional screening nursery for BLS providing data for growers of the relative resistances in commercial wheat varieties. Although host resistance is critical to disease control, there is no immunity to BLS and additional control options would be advantageous to growers. This project proposes research aimed at developing additional tools that can be used by the grower in the management of BLS. In conjunction with the use of varieties with improved resistance, these tools can provide additional options to the grower in the management of this economically important disease.

Research methods:

1. Validate molecular assays as tools to rapidly and reliably identify *Xtu* in plant tissues and soil samples. In 2021 we tested molecular assays (LAMP and PCR) to identify *Xtu* in wheat seeds. These assays have several practical applications, including identifying seed lots that are free of, or minimally infested with, *Xtu*. Our goal was to be able to effectively detect contaminated seed lots, such as samples submitted by growers to the UMN Plant Disease Clinic or seed that the breeding programs ship internationally. Clean seed may prove an effective way to reduce the risk of disease in high value seed lots, including foundation seed and seed exchanged between countries such as the UMN breeding program's winter increase in New Zealand. In this project we plan to validate the molecular tools (LAMP and PCR assays), developed to identify *Xtu*-contaminated seed lots, and to detect the pathogen in other matrices including plant tissues and soil. The two assays (LAMP and PCR) are based on the detection of DNA. We have still not determined all the places where the bacterium may be surviving between growing seasons, and crop debris and soil have yet to be studied as potential pathogen reservoirs. The ability to detect the pathogen is key to elucidating its full lifecycle and identifying where it survives between growing seasons may suggest additional control options. We also plan to adapt a qPCR protocol that detects and quantifies viable *X. translucens* cells. Such a method has been developed for the related bacterium, *Xanthomonas hortorum* (Temple et al. 2013). We will use seed from the 2022 field season Minnesota on-farm variety trials to validate the detection of *Xtu* in seed using the methods outlined above. Jochum Wiersma (UMN), who conducts these trials, has agreed to alert our research group to locations with significant naturally occurring BLS. We will travel to these sites, rate BLS and obtain seed following harvest from select varieties (including both BLS susceptible and BLS resistant varieties) from locations with high and low disease pressure. In addition to validating the best of the three molecular tests (LAMP, PCR and/or qPCR) available, we will also isolate bacteria from these samples using dilution plating to confirm the presence of viable bacterial cells in the test samples.

2. Determine where in the wheat seed the bacterium is surviving. We plan to continue the work we started in 2020 examining how *Xtu* colonizes wheat seed to determine where in the wheat seed the pathogen is residing. If *Xtu* lacks the ability to enter the interior of the seed, antibacterial seed treatments, such as copper compounds, or physical seed

treatments such as the applications of heat, may prove useful in reducing seed transmission of the pathogen. Seed will be dissected into major components (bran and endosperm) and qPCR will be used to quantify the bacterial load in each component. This work should confirm if *Xtu* is inside the wheat seed and associated with the embryo, or if *Xtu* is surviving only on the seed coat.

3. Determine how long the bacterium is surviving in association with wheat seed. In addition to understanding where in the seed *Xtu* is surviving, we plan to use the qPCR assay to quantify bacterial cells in seed obtained from the on-farm yield trials, and/or following artificial inoculation, at regular intervals after harvest to determine how long the bacteria associated with seed remain viable. An understanding of the duration of viability would also aid in developing recommendations for seed handling and treatments aimed at reducing the risk of BLS in a subsequent crop.

4. Examine the efficacy of seed treatments in reducing *Xtu* in association with seed. Physical treatments, such as heat (wet or dry), are reported to be effective in killing the bacteria in association with seed. In 2020 we started to examine the role of infected seed in the epidemiology of BLS. In 2022 we plan to examine the use of wet and dry seed treatments on naturally infected seed, obtained from the Minnesota on-farm variety trials, with the goal of reducing *Xtu* in association with seed. However, physical seed treatments, like chemical treatments, are associated with decreased seed germination and are also most effective on bacteria located close to the seed surface. Seed treatments are thus likely to be most effective if combined with a rapid and reliable test that determines the level of infestation and will likely be limited to use in high value seed lots. Recovery of the bacterium, using dilution plating before and after treatment, will be used to determine the efficacy of the treatments. Germination tests will also be conducted to determine the impact of the treatments on seed viability.

5. Conduct field trials in collaboration to examine the efficacy of commercial foliar treatments on BLS. We plan to undertake field trials at two Minnesota locations to examine commercially available copper-based (Champ, Cuprofix Ultra, Kocide, Oxide 5.0, and MasterCop) and biological treatments (Actinovate [Actinomyces] and Aviv [Bacillus]) on BLS development in wheat. In addition, non-inoculated and inoculated & untreated control treatments will be included. The trials will be inoculated, and BLS development will be assessed visually, and yield and test weight will be determined. Our first year of testing, conducted in 2021, showed little impact of copper-based treatments however the dry season was generally unfavorable for BLS development and in 2022 we have expanded the number of compounds we are examining.

Timeline for completion: The goal of our research is to provide growers tools to improve the control of BLS in wheat. We work closely with the plant breeding programs and our efforts directed toward genetic improvement in wheat resistance are ongoing and will extend beyond the life of this project. We anticipate the studies objectives in this proposal; to understand the localization of *Xtu* in wheat seed and the efficacy of seed treatments will be completed in 2022. Some laboratory-based components of our work started in 2020 were delayed due to pandemic-related campus closures, however we anticipate being able to complete all the work outlined in the 2022 proposal by December 2022.

Outreach plan: The information obtained through this work will be disseminated through the publication of scientific journal articles. Our findings will also be published in formats targeted to growers such as disease factsheets and presented in person at annual events attended by growers and Ag professionals including Prairie Grains, The Best-of-the-Best, the Advanced Crop Advisors Workshops, and the annual NWROC field day.

List other current or pending funding sources for this project: Current (2021) MNWR&PC project: $58,608

Research group (other collaborators not listed as PIs): Jochum Wiersma & James Anderson (UMN), Jonathon Jacobs (The Ohio State University), Andrew Friskop (NDSU), and Zhaohui Liu (NDSU)

Relationship to past projects and research conducted by you or others in the region: The goals of this research proposal follow as logical developments from the previously funded projects. Work screening materials for reaction to BLS from the UMN, NDSU and SDSU breeding program is funded through the MN Small Grains Initiative and for private wheat breeding programs on a fee per entry basis.

Minnesota Wheat Research and Promotion Council

RESEARCH PROJECT PROPOSAL BUDGET

Project Title: Research on Bacterial Leaf Streak of Wheat

Principal Investigator(s) / Project Director(s) Ruth Dill-Macky	Funds Requested For Year 1 (2022)	Year 2 (2023)	Year 3 (2024)
A. Salaries and Wages	$ 28,360	$	$
1. Co-principal Investigator(s)			
2. Senior Associates			
3. Research Associates – Post Doctorate			
4. Other Professionals			
5. Graduate Students	28,360		
6. Prebaccalaureate Students			
7. Secretarial - Clerical			
8. Technical, Shop and Other			
B. Fringe Benefits	9,322		
C. Consulting and Professional Services			
D. Supplies and Services	12,618		
E. Travel	1,500		
F. Sub-Contracts			
G. Repairs & Maintenance			
H. Rentals & Lease	1,200		
I. Other Expenses			
TOTAL AMOUNT OF THIS REQUEST (per year)	**$ 53,000**	**$**	**$**