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

PI# 2 Name:

PI# 3 Name:

4. PI #1 BUSINESS ADDRESS
Department of Plant Pathology
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University of Minnesota
St. Paul, MN 55108

5. PROPOSED PROJECT DATES (calendar years)
January 1, 2021 – December 31, 2021

Note: Research Reports are Due November 15th of Each Year

6. TOTAL PROJECT COST

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 (LAMP and PCR) as tools to rapidly and reliably identify *Xanthomonas translucens* pv. *undulosa* (*Xtu)* on wheat seed, plant tissues, and soil samples for use in a commercial setting.
2. Determine where in the wheat seed the bacterium is surviving to aid understanding of seed transmission.
3. Examine the efficacy of seed treatments in reducing *Xtu* in association with seed to determine if seed is important in driving bacterial leaf streak (BLS) development in wheat crop planted using infested seed.
4. Conduct field trials to examine the efficacy of commercial foliar treatments (copper and biological control agents (BCA) / and timing of BCA applications) on the control of BLS.
5. Examine the baseline sensitivity of the Minnesota *Xtu* population to copper.

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 Of Principal Investigator	**Date**	**Phone Number**
Ruth Dill-Macky	11-January-2021	651-399-0947 (cell)

Signature Of Authorized Representative	**Title**	**Date**
Nicolas Allyn	**Principal Grants and Contracts Administrator**	**1/12/2021**

Address Of Authorized Representative	**Phone Number**
Nicolas Allyn, Office of Sponsored Projects Administration 450 McNamara Alumni Center, 200 Oak Street SE, Minneapolis, MN 55455-2070	**612-624-5599**

Project Title: Research on Bacterial Leaf Streak of Wheat

Importance of this project to the profitability of wheat producers:
Bacterial leaf streak (BLS) of wheat continues to cause significant economic damage to wheat in Minnesota. The ultimate goal of the project is to deliver economic disease control measures for this disease to growers. Our proposed work will explore the biology of the BLS pathogen with the aim of uncovering avenues of disease control that compliment host resistance and to examine disease management strategies that could complement host resistance. Outcomes, of practical value to the wheat grower, stem from our work understanding the pathogen. The development and validation of tools for the identification and/or quantification of BLS infection within seed lots, crop debris and soil are proposed in this project. These tools may be useful in identifying seed lots and specific fields that are at higher risk of BLS. In addition, we propose to examine the efficacy of seed treatments in disinfesting seed infected by the pathogen. In response to inquiries from the wheat industry we will undertake field trials in conjunction with NDSU to examine the efficacy of foliar sprays (chemical and biological) in reducing BLS development. As *Xanthomonads* are known for their ability to rapidly develop resistance to copper, we will examine the sensitivity of strains in our collection to provide an understanding of the baseline sensitivity of this pathogen to copper. In conjunction with the use of varieties with improved resistance, the development of these tools will provide additional options to the grower in the management of this economically important disease.

Procedures:
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 ineffective against bacterial pathogens thus host resistance provides the principle disease control strategy. Although host resistance is critical to disease control there is no immunity to BLS. This project proposes research aimed at developing additional tools that can be used by the grower in the management of BLS.

1. Validate molecular assays (LAMP and PCR) as tools to rapidly and reliably identify Xtu on wheat seed, plant tissues, and soil samples.

We plan to validate molecular tools (PCR and LAMP assays) that have been developed to identify *Xtu* and determine if these can be used to identify *Xtu*-contaminated seed lots. These assays will be corroborated using dilution plating that will determine the bacterial load and confirm if the bacterial DNA detected using the molecular assays is indeed detecting viable bacteria. Initially, and starting in Fall 2020, we will use seed from the 2020 field season to complete the preliminary work. In 2021 we will obtain seed from the Minnesota on-farm variety trials to validate the usefulness of this data. Jochum Wiersma (UMN, NWROC) will alert our research group to trial 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 resistance varieties). We will use the seed to validate tools for the detection of *Xtu* in seed. Specifically we plan to validate a loop-mediated isothermal amplification (LAMP) assay (Langlois et al. 2017) for the identification of a small group of *X. translucens* pathovars (specifically the pathovars; *undulosa*, *translucens*, and *secalis*). Our preliminary studies indicate that while it is an effective tool there are some strains (specific sequence types) of *Xtt* that can evade detection using this technique. The second technique that we will validate uses polymerase chain reaction (PCR) technology. Specifically we will validate two primers sets developed by Jonathan Jacobs (The Ohio State University) that are specific for *Xtu* and *Xtt*, respectively. As these two assays are based on the detection of DNA we will also isolate bacteria from these samples using dilution plating to confirm the presence of viable bacterial cells in the test samples. Once we have a tool that works reliably on seed we will adapt these tests to detect the pathogen in other matrices (plant tissues and soil). Our goal is to be able to effectively detect contaminated seed lots, plant debris, and soil in commercial settings, such as samples submitted by growers the UMN Plant Disease Clinic or for seed lots that breeding programs ship internationally.

2. Determine where in the wheat seed the bacteria are surviving.

In this goal we will determine where in the wheat seed the bacteria (*Xanthomonas translucens* pv. *undulosa*) are surviving. Our colleague Jonathon Jacobs (OSU), has evidence that *Xanthomonas translucens* pv. *translucens* (*Xtt*), the bacterium that causes BLS in barley, is a vascular pathogen able to move in the xylem vessels that carry water in the host plant. It is thought that *Xtu* (the wheat pathogen) is not vascular, meaning it is not able to colonize the vascular tissues of wheat. It has long been assumed that both *Xtu* and *Xtt* bacteria are seed-borne and that these pathogens were similar in the their ability to colonize grain. In the project we propose for 2021, we plan to examine how *Xtu* colonizes wheat seed to determine if the *Xtu* bacterium is surviving inside and/or on the surface of wheat seed. This information will improve our understanding of *Xtu* survival in seed and provide an indication of the role of seed in driving BLS epidemics. If *Xtu* lacks the ability to enter the interior vascular tissues of the seed, antibacterial seed treatments, such as copper compounds, or physical seed treatments such as the applications of heat, may be useful in reducing seed transmission of this pathogen. We plan to use strains of the bacterium tagged with a fluorescent protein that will allow us to visualize the bacterium in association with the wheat seed. Once we have inoculated plants with the tagged stain we will use microscopy to visualize the bacterium in the seed. This work should confirm if *Xtu* is inside the wheat seed and associated with the embryo, or *Xtu* is surviving only on the seed exterior.

3. Examine the efficacy of seed treatments in reducing Xtu in association with seed to determine if seed is important in driving BLS development in a subsequent wheat crop.

To determine where in the wheat seed the bacteria are surviving we will use naturally infested seed to examine the efficacy of seed treatments in reducing *Xtu* in association with seed. In 2021 we plan to expand this work to validate

testing using naturally infected seed from the Minnesota on-farm variety trials and to examine the efficacy of seed treatments in reducing *Xtu* in association with seed. This objective builds on work we recently completed that provided us an understanding the diversity of the pathogen. Multi-locus sequence typing (MLST) provides a tool to identify strains of the Xtu population that should enable us to determine the role of seed in the epidemiology of BLS. MLST allows us to recognize sequence types of the pathogen based on the unique sequence of four genes. We plan to select a naturally occurring isolate of *Xtu*, with a known sequence type, inoculate seed, using seed infiltration, and then recover bacteria from the developing seedling/plant, using dilution plating, and identify the recovered strains using MLST. Recovery of the same sequence type that we used to inoculate the seed would provide evidence that seed is contributing to the development of BLS in the crop grown from infected seed. If we can demonstrate this then the identification of infested seed lots would be critical in reducing the carry forward of the disease from one season to the next and would be an indication that seed treatments may be efficacious in disease control. We plan to use seed from a select variety obtained from sites of the MN variety trials with varying BLS infections, treat the seed using a period of dry heat (162°F for seven days) in conjunction with dilution plating before and after treatment to determine the efficacy of the treatment, followed by recovery of the bacterium from the developing seedling/plant tissues. Germination tests will also be conducted to determine the impact of the treatment on seed viability.

4. Conduct field trials in collaboration to examine the efficacy of commercial foliar treatments (copper and biological control agents) on the control of BLS.

We plan to undertake field trials at four locations (two in MN and two in ND). The MN locations will be St Paul and Crookston while the North Dakota trials will be conducted by Andrew Friskop (NDSU). The replicated trials with examine five commercially available treatments (a.i. copper, applied at flag leaf) and two biologicals (*Streptomyces* and *Bacillus*; applied both early [at the 3-4 leaf stage] and at the flag leaf stage). The treatments will be applied to a popular variety in the middle of the range with respect to reaction to BLS. In addition an untreated control treatment will be included. The trials will be inoculated, BLS development will be assessed visually, and yield and test weight examined in the harvest plots.

5. Examine the baseline sensitivity of the Minnesota Xtu population to copper.

Copper is the most widely recognized bactericide but is problematic both because of it's toxicity to the plants and reports of the development of copper resistance strains of *Xanthomonas* in a number of pathosystems. Using our collection of *Xtu* we will determine the baseline sensitivity of the pathogen population to copper. We have a sizable *Xtu* collection, and we know that the *Xtu* population on wheat in Minnesota is quite diverse and we have isolates that are representative of that diversity. We will select strains that are representative of the population of *Xtu* found in association with wheat and determine the baseline sensitivity by growing the pathogen on media containing copper, at a range of concentrations. Information on the current sensitivity of the *Xtu* population to copper should allow us to determine if the pathogen already has some resistance to copper and may thus be able to readily adapt to the deployment of copper-based products in the control of BLS. Given the history of many closely related bacteria developing rapid resistance to copper we think this is advisable ahead of recommending any copper-based treatments in the control of BLS.

Regional linkage to other research activities:

Dr. Shakaut Ali (SDSU) and Dr. Zhaohui Liu (NSDU) host the SD and ND locations of the BLS cooperative nursery (BLSCN). Dr Andrew Friskop (NDSU) will conduct the two trials outlined in Objective 4.

List current or potential other funding sources for this project:

Minnesota Small Grains Initiative project titled “Evaluation of small grains cereals for multiple disease resistance and mycotoxins.” supports efforts to phenotyping wheat and barley for reaction to ten diseases. ($80,791 = FY20/21 funding)

Research Group:

Ruth Dill-Macky is the PI who will lead the research outlined in the proposal. Kristi Ledman, a graduate student, will work on the BLS objectives outlined in this proposal. Rebecca Curland, a Research Professional has expertise in bacteriology.

Relationship to past projects:

The goals of this research proposal follow as logical developments from the previously funded projects.

Estimate the budget requirements:

Wages and fringe benefits: $47,938 for the support of a graduate student ($26,281 annual salary, $16,427 tuition, FB 19.9% [$5,230]) to work on BLS. Materials and Supplies: $8,470 for materials needed for bench work to complete laboratory and field studies. Other Direct Costs: $1,200 for field and greenhouse costs. Travel: $1,000 is requested to cover travel to MN on-farm yield trials for disease assessment and sampling.

References:

Curland, R.D., Gao, L., Bull, C.T., Vinatzer, B., Dill-Macky, R., Von Eck, L., and Ishimaru, C.A. 2018. Genetic diversity and virulence of wheat and barley strains of *Xanthomonas translucens* from the Upper Midwestern United States. *Phytopathology*, 108:443-453.

Langlois, P. A., Snelling, J., Hamilton, J. P., Bragard, C., Koebnik, R., Triplett, L. R., Blom, J., Tisserat, N. A., and Leach, J. E. 2017. Characterization of the *Xanthomonas translucens* complex using draft genomes, comparative genomics, phylogenetic analysis, and diagnostic LAMP assays. *Phytopathology* 107:519–527.

Ledman, K.E., Curland, R.C., Ishimaru, C.A., and Dill-Macky, R. (2020). *Xanthomonas translucens* pv. *undulosa* identified on common weedy grasses in naturally infected wheat fields in Minnesota. *Phytopathology (First Look* 23 Nov 2020).

Tubajika, K. M., Tillman, B. L., Russin, J. S., Clark, C. A., and Harrison, S. A. 1998. Relationship between flag leaf symptoms caused by *Xanthomonas translucens* pv. *translucens* and subsequent seed transmission in wheat. *Plant Dis.* 82:1341–1344.