Minnesota Wheat Research and Promotion Council
FULL RESEARCH PROPOSAL For Crop Year 2025

Project Title: Optimization of Bacterial Leaf Streak Screening Nurseries for Hard Red Spring Wheat in Minnesota
Principle Investigator (PI): Rebecca Curland
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Additional Investigator(s): Ruth Dill-Macky and James Anderson
Organization: University of Minnesota
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Project Period: January 1, 2025 – December 31, 2026
Estimated cost: $96,408 (for two years)

Abstract
Bacterial leaf streak (BLS), caused by *Xanthomonas translucens*, has become a significant disease in wheat-growing regions, including Minnesota. The disease leads to reduced yield and grain quality, making it a priority concern for wheat growers. As no control methods have been identified for BLS, efficient screening methods to identify resistant varieties are critical for mitigating the impact of BLS on wheat production. The objectives of this project are:

1) To evaluate differences in disease response to recent *Xanthomonas translucens* isolates across 12 genotypes of hard red spring wheat to determine if resistance in wheat is effective to the current pathogen population.
2) To examine the impact of inoculation timing on disease severity ratings, particularly to ensure that late-heading varieties are properly evaluated.

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) poses a significant economic threat to wheat production in Minnesota, making the development of reliable and efficient screening methods essential for breeding wheat varieties with improved resistance. Over the past 15 years, our program has established reproducible screening techniques; however, it is crucial to assess whether the isolates in use are current and reflect the genetic diversity identified in recent studies of *Xanthomonas translucens*. We have a large collection of *Xanthomonas translucens* pv. *undulosa* strains that were isolated from BLS-infected wheat in Minnesota from 2009-2024. In our previous work, we examined the genetic diversity of strains collected between 2009-2017 (Curland et al. 2018, Curland et al. 2020, Ledman et al. 2021). However, it is unclear which *X. translucens* pv. *undulosa* lineages are present in more recent outbreaks (2019-2024). We will use genome sequence data to determine which bacterial genotypes are present in BLS infections of commercial wheat crops from the past five years and test a subset of these strains in field trials using a variety of wheat lines.
We will also investigate how inoculation timing affects disease severity. We have observed a negative correlation between heading date and disease severity. By testing inoculation timing in field trials on both early- and late-heading wheat varieties, we aim to ensure our methods accurately capture variations in disease severity. This research will allow us to optimize our screening nurseries to provide improved data for breeding programs, ultimately accelerating the development of BLS-resistant varieties. Ultimately, the outcomes of this project will benefit wheat breeders and producers in Minnesota by reducing the prevalence of BLS and enhancing wheat yields.

Research Methods
Evaluation of strains for screening nurseries. Our collaborator, Andy Read (USDA-ARS), has agreed to sequence the genomes of 12 strains collected in Minnesota over the past five years, generating genotypic data to help us select two strains for use in our field trials. Field trials will be conducted during the 2025 and 2026 growing

seasons. Twelve wheat varieties, selected for genetic diversity, differences in heading date (early and late) and varying susceptibility to BLS, will be planted in two-row plots with four replications per treatment. Three inoculation treatments will be tested, including two genetically distinct *X. translucens* pv. *undulosa* strains that represent the two phylogenetic clades that have been identified in the pathogen population (Curland et al. 2020) and a mixture of both strains. Treatments will be arranged in blocks, separated by buffer rows to prevent cross-contamination. Plots will be inoculated at the flag leaf stage using a gas-powered backpack sprayer with bacterial suspensions. Disease severity will be assessed two- and three-weeks post-inoculation.

Determination of optimal inoculation timing. The same panel of wheat varieties will be utilized for this field trial. Two-row plots will be planted with timing treatments arranged in blocks separated by buffer rows. Each variety will be replicated four times within each treatment. Three inoculation timings will be tested: jointing, flag leaf, and anthesis. The trial will be planted twice each year to represent one early and one later planting date, spaced two weeks apart. Plots will be inoculated using a gas-powered backpack sprayer with a bacterial suspension of strain CIX40, which has been used in our screening nurseries since 2013. Disease severity will be assessed two- and three-weeks post-inoculations.

Outline the timeline for completion:

The project will be conducted over a two-year period:

- **Year 1 (2025)**: Recent *Xanthomonas translucens* pv*. undulosa* isolates will be sequenced and two isolates representing diverse genetic clades will be selected for field trials. Field trials will be established to observe isolate by wheat genotype interaction and to test inoculation timing.
- **Year 2 (2026)**: Data from the first year will inform adjustments, and replications of the previous year's experiments will be completed. Data will be analyzed and summarized.

What methods, if any, will be used to disseminate your research findings out to the greater public, beyond the final report due to Minnesota Wheat Research and Promotion Council:

We will present our findings at events such as the NWROC's annual Crops and Soils Day in Crookston, Prairie Grains Conference, the Small Grains Update, and at relevant scientific meetings such as the annual Regional Bacterial Leaf Streak Research Meeting.

List potential collaborators or co-investigators you may consider inviting to participate:

Andrew Read's (USDA-ARS) group will prepare and submit recently collected *Xanthomonas translucens* strains for sequencing, and Milton Drott's (USDA-CDL) lab will assemble and annotate sequences.

Estimate the budget requirements:

Wages and fringe benefits: $91,508 (40% salary and fringe for 2 years) for Rebecca Curland, Research Scientist who will oversee the project. $2,000 for undergraduate technician pay to help manage the field plots.

Materials and Supplies: $500 annually for lab supplies for bacterial media preparation and inoculum production.

Field plot rental: $250 annually to rent space in the University of Minnesota's St. Paul Research Plots.

Travel: $500 per year to attend field day events

List sources and amounts of additional funding for this project, and indicate if they have committed to provide funding or if you have requested funding:

Andrew Read's (USDA-ARS) group will cover the cost of sequencing recently collected *Xanthomonas translucens* strains. Andrew Read and Milton Drott do not request salary to support research personnel as they have funding support from additional sources.

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.

Curland, R.D., L. Gao, L., Hirsch, C.D., and Ishimaru, C.A. 2020. Localized genetic and phenotypic diversity of *Xanthomonas translucens* associated with bacterial leaf streak on wheat and barley in Minnesota. Phytopathology 110:2, 257-266.

Ledman, K.E., Curland, R.D., Ishimaru, C.A., and Dill-Macky, R. 2021. *Xanthomonas translucens* pv. *undulosa* identified on common weedy grasses in naturally infected wheat fields in Minnesota. Phytopathology 111:1114-1121.

Minnesota Wheat Research and Promotion Council
RESEARCH PROJECT PROPOSAL BUDGET

Project Title: Bacterial Leaf Streak (BLS) resistance in Minnesota Germplasm

Principal Investigator(s) / Project Director(s) Rebecca Curland	Funds Requested For Year 1 (2025)	Year 2 (2026)	Year 3 (2027)
A. Salaries and Wages			$
1. Co-principal Investigator(s)	$33,000	$33,990	
2. Senior Associates			
3. Research Associates – Post Doctorate			
4. Other Professionals			
5. Graduate Students			
6. Prebaccalaureate Students	$1,200	$1,200	
7. Secretarial - Clerical			
8. Technical, Shop and Other			
B. Fringe Benefits	$12,078	$12,440	
C. Consulting and Professional Services			
D. Supplies and Services	$500	$500	
E. Travel	$500	$500	
F. Sub-Contracts			
G. Repairs & Maintenance			
H. Rentals & Lease	$250	$250	
I. Other Expenses			
TOTAL AMOUNT OF THIS REQUEST (per year)	**$ 47,528**	**$ 48,880**	**$**