Minnesota Wheat Research and Promotion Council
CROP YEAR 2013 RESEARCH REPORTING FORM
Form Due November 15, 2013

1. PROJECT TITLE
A Coordinated Research Plan to Address Bacterial Leaf Streak

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7. AMOUNT OF GRANT $37,500

8. PUBLICATIONS

Dill-Macky, R. 2010. Research Update for Agricultural Professionals, Crookston MN, January 14, 2010.
Kandel, Y.R., Osborne, L.E., Glover, K.D., and Tande, C.A. 2010. Diagnosis and Appraisal of Bacterial Leaf Streak Disease Severity in Wheat. Proc. SD Acad. Sci. 89:258.
Kandel, Y.R., Osborne, L.E., Glover, K.D., and Tande, C.A. 2010. Response of hard red spring wheat germplasm to the bacterial leaf streak pathogen (*Xanthomonas campestris* pv. *translucens*). Phytopathol. 100:S59.
Kandel, Y.R., Osborne, L.E., Glover, K.D., and Tande, C.A. 2011. Yield loss in spring wheat due to disease caused by *Xanthomonas campestris* pv. *translucens*. Phytopathol. 101:S87.
Kandel, Y. R., K. D. Glover, and L. E. Osborne. 2012. Effect of inoculation method, inoculum concentration, and plant growth stage on development of wheat bacterial leaf streak. Phytopathology 102:S4.61.
Kandel, Y. R., K. D. Glover, and L. E. Osborne. 2012. Relationship between the greenhouse and field reactions of spring wheat genotypes to Bacterial leaf streak pathogen (*Xanthomonas campestris* pv. *translucens*). Phytopathology 102(Suppl. 5), No.9:S5.5.
Kandel, Y. R., L. E. Osborne, K. D. Glover, and C. A. Tande, 2011. Yield loss in spring wheat due to disease caused by *Xanthomonas campestris* pv. *translucens*. Phytopathology 101 (6):S87.
Kandel Y.R., K.D. Glover, C.A. Tande, and L.E. Osborne. 2012. Evaluation of spring wheat germplasm for resistance to bacterial leaf streak caused by *Xanthomonas campestris* pv. *translucens*. Plant Disease. 96:1743-1748.
Kandel, Y.R. 2013. Germplasm evaluation for bacterial disease resistance and mapping QTL conferring resistance against *Xanthomonas campestris* pv. *translucens* in wheat. PhD dissertation, South Dakota State University. Brookings, SD.
Results and progress were presented at several grower meetings in the spring and summers of 2011 and 2012.

9: EXECUTIVE SUMMARY

Research Question:

Bacterial Leaf Streak (BLS) of wheat has been evident in Minnesota, North Dakota and South Dakota in the past five cropping seasons. While generally sporadic in nature, the levels of BLS in recent years have been of concern for wheat production. Bacterial leaf streak, caused by *Xanthomonas translucens* pv. *undulosa,* results in distinctive symptoms on leaves and glumes. Although BLS can be severe, the impact on yield and grain quality and disease control options are not well understood. As with most plant diseases, genetic resistance likely offers the most economic and effective control for producers. From 2010 to 2012, the Minnesota Wheat Research and Promotion Council has funded cooperative research among MN, ND and SD to conduct preliminary research on BLS. Substantial progress has been made over the course of these projects and our 2013 project proposed to continue this research to complete the initial phase of our research on BLS and to start a transition to a new phase of the research. The data generated in 2013 has provided valuable information that will be using to shape future control efforts, including breeding for resistance. The six principle objectives of the 2013 project were:

1) Develop techniques for producing inoculum and inoculating plants in the greenhouse and field
2) Establish if BLS is of economic importance
3) Examine the structure of the pathogen population
4) Develop a cooperative regional BLS screening nursery
5) Examine the influence of foliar fungicides on BLS development

and

6) Strengthen our collaborative research effort on BLS

Results:

1) UMN continued to examine field based inoculation methods for establishing screening nurseries for BLS in 2013. The data from these field experiments have provided valuable insights on the methods needed for the establishment of reliable and repeatable field-based assessments. From our experiences in 2013, it was evident that we still need to refine the technique(s) we use to apply inoculum in the field. In 2013 we observed rather patchy development of BLS symptoms in many inoculated field plots. The patchy disease pattern presumably resulted from the limited spread of symptoms beyond the tissues that received a direct application of inoculum. While we have observed this phenomenon in previous years it was very pronounced in 2013. The addition of carborundum, a fine abrasive, to the inoculum appeared to aid inoculation in 2011 and 2012. In some 2013 trials though it appears that carborundum had had little or no effect on disease development. The efficacy of inoculations appears to be impacted by environmental conditions, more than by the growth stage(s) of the plants at the time of inoculation. Needle inoculations, while producing high levels of BLS in individual plants, have been rejected as an inoculation option as we were again unable to demonstrate any spread of symptoms to adjacent plants in 2013. BLS was however successfully established on dryland sites in 2013, confirming our earlier findings indicating that screening nurseries can be conducted without irrigation.

In 2013 UMN continued to evaluate wheat lines for susceptibility to BLS under greenhouse conditions. Since the inception of this project in 2010, advanced lines in the Minnesota wheat breeding program (2010 AY1, 2011 AY1, 2012 AY1 and a collection of Jim Anderson's advanced breeding materials) have been screened for their reaction to BLS in greenhouse experiments. The wheat lines were each screened with two *X. translucens* isolates via leaf infiltration of the flag leaf. Full screenings were performed in duplicate. Lesion length and area measurements were digitally recorded for each lesion. Analysis indicates that lesion length and lesion area are highly correlated, therefore in future studies only lesion length measurements will be determined for a more time-efficient rating process. Data analysis is currently in process and will be used to compare greenhouse ratings with field ratings from the 2010-2013 field seasons.

2) In 2013, UMN examined the impact of BLS on yield in field experiments at St. Paul and Crookston. Yield losses were readily observed in the wheat cultivars examined with disease severities of ca. 15% resulting in yield losses approaching 30% (see Appendix 1).

3) UMN observed all isolates (collected 2009-2012 and representing strains from both wheat and barley) and other *X. translucens* pathovars (*poae*, *cerealis*, *phleipratensis* and *secalis*) to be pathogenic in RB07 and Blade in greenhouse screenings. Virulence of isolates varied widely. RB07 was more susceptible than Blade overall, but there were several isolates which were more virulent on Blade than RB07. These results indicate that strain-genotype interactions and variations in isolate virulence should be considered in breeding efforts. Additionally, as all *X. translucens* pathovars from wheat and barley were shown to be virulent on wheat, it is necessary to confirm the identity of *X. translucens* isolates present in Minnesota to ensure that materials are screened for all pathovars present in the region.

MLST (multi locus sequence typing) primer sets for four genes were identified and PCR protocols were refined to produce optimal amplification. A subset of 24 isolates representing a variety of locations, including type strains from six different *X. translucens* pathovars were selected for preliminary MLST analysis. All four loci were successfully amplified in the 24 isolates and clear sequence data was generated. In March 2013, Rebecca Curland (UMN Research Fellow) traveled to Dr Carolee Bull's laboratory in Salinas, CA and received training in MLST analysis. MLST of UMN's entire isolate collection (including 12 shared isolates from SDSU) is currently in progress. Greenhouse experiments during winter 2013-2014 will confirm phenotypic data for all isolates and will be integrated into MLST phylogenies.

4) We (UMN, SDSU and NDSU) undertook the screening of a cooperative nursery (BLSCN) consisting of 114 wheat lines sourced from the UMN, SDSU, NDSU, Limagrain, WestBred and Agripro spring wheat breeding programs. This cooperative nursery (BLSCN) was planted at four locations - Saint Paul, Crookston, Brookings and Fargo. We successfully obtained data on the relative response of these entries at all locations. The BLS rankings were significantly correlated between field sites (see Appendix 2).

UMN also undertook a screening of advanced breeding lines (2013 AY1) in the greenhouse and at three field locations in MN. The Lamberton nursery was essentially lost as it was planted too late due to the late spring thaw, but at the other locations (St Paul and Crookston) variation in host susceptibility to BLS was observed. In addition to screening of advanced lines, several populations selected by Dr. Jim Anderson, which were thought to be segregating for BLS, were screened in the 2013 BLS field nurseries and the data provided to Dr. Anderson.

A successful nursery, screening a broad range of wheat germplasm, was also conducted by UMN in cooperation with Dr. Blake Cooper (Limagrain) at Foxhome MN. While hail damaged this trial shortly before harvest we trust that the analysis of the disease ratings of this trial (yet to be completed) and grain weight data will provide valuable information on both the expected losses from BLS and the impact of fungicide applications on disease development.

NDSU conducted disease evaluation for a total of 220 entries in a BLS field nursery that was established near the campus in Fargo. While 114 entries were from the BLSCN, the remaining 106 entries consisted of the major spring wheat cultivars currently grown in ND and the surrounding region as well as the elite lines from NDSU spring wheat breeding program. The susceptible checks showed severe BLS symptom on flag leaves indicating that we obtained high levels of disease pressure in Fargo nursery. Differences in disease severity were clearly observed among the entries. We also observed that durum wheat cultivars were generally less susceptible than hard red spring wheat (HRSW) cultivars and only one HRSW cultivar ‘Barlow’ was moderately susceptible. Among the elite lines evaluated, most lines are susceptible and only three were identified as having similar levels of resistance as the resistant checks.

5) In the 2013 wheat growing season, SDSU conducted a preliminary field experiment to determine the effect of *X. transluceens* pv. *undulosa* alone, a mixture *Pyrenophora tritici-repentis* (tan spot) and *X. transluceens* pv. *undulosa,* and a mixture of *P. tritici-repentis, X. transluceens* pv. *undulosa* and a fungicide application on bacterial leaf streak development. Field plots of two BLS susceptible hard red spring wheat cultivars, Briggs and Select were established on May 13, using randomized complete block with split plot arrangement with three replications. The plot size was 5 x 20 feet. Strips (10 x 20 feet) of Steele-ND cultivar were planted between the plots and between the replications (20 x 20 feet) to avoid/decrease the chances of cross contamination among the treatments. There were 5 treatments in the experiment.

- Treatment 1: inoculated with *X. transluceens* pv. *undulosa* only
- Treatment 2: inoculated with *X. transluceens* pv. *undulosa* and *P. tritici-repentis*
- Treatment 3: inoculated with both pathogens and sprayed with “Prosaro” 12 hours post-inoculation
- Treatment 4: inoculated with *P. tritici-repentis* only
- Treatment 5: plants were sprayed with water only and served as a check

The inoculations were conducted at around 9:00 pm and the fungicide was applied 12 hours post-inoculations. The inoculations were done when the plants were at heading stage on June 28. BLS disease data was collected after 18-days of inoculations using rating scale 1-9. A decreasing trend in BLS development was observed among treatments T2 and T3 as compared to T1. BLS disease severities of 7.7, 6.3, and 6.0 were observed in cultivar Select in T1, T2, and T3, respectively. The disease severity observed in Briggs was 5.7, 4.7, and 4.0 in T1, T2, and T3, respectively. Although the disease severity in cultivar Briggs was lower than in Select the treatments effect trend was similar in both cultivars (see Appendix 3). The results indicate that the mixed pathogens infection on leaves may be providing some kind of competition for space occupation. Contrary to our expectation, the BLS disease severity was lower in fungicide treated plots (T3) as compared to untreated plots (T1). We plan to repeat this experiment under both field and greenhouse conditions.

6) We have organized conference calls and face-to-face meetings and site visits of the researchers involved in this project as necessary to coordinate the activities of this project and strengthen the collaborative ties among the scientists involved in this project.

Application/Use:

Our ability to generate the disease is necessary to determine the impact of the disease on wheat, evaluate variability in the pathogen, and develop disease control strategies, including resistance. Developing resistant wheat germplasm will rely on effective and efficient screening methods both to identify sources of resistance and to introgress resistance into adapted germplasm. In 2013 we confirmed the efficacy of techniques developed to facilitate the establishment of reliable and repeatable assessment of BLS.

In 2013 we gained additional evidence of the significant economic impact of BLS. This demonstrates the importance of this disease in wheat production and justifies further research to develop a better understanding of BLS in wheat and effective management strategies.

We have demonstrated that there is considerable genetic variability in this pathogen. We still need to determine the biological importance of this variation to disease development and control.

The screening nurseries have provided information on the response of commercially available cultivars and advanced breeding lines to BLS, which enables wheat growers and breeding programs to make decision on variety or line selection.

Materials and Methods:

1) **Develop techniques for producing inoculum and inoculating plants in the greenhouse and field.** Our ability to generate BLS is necessary to determine the impact of the disease on wheat, evaluate variability in the pathogen, and develop disease control strategies including resistance, which relies on screening to identify sources of resistance that are subsequently introgressed into adapted germplasm.
   - UMN, SDSU: both programs worked with spring wheat and SDSU also worked with winter wheat in developing screening techniques in the greenhouse and field.
   - UMN and SDSU: UMN and SDSU each have two years of field data but after a challenging 2012 field season we planned to undertake additional experiments in 2013 to be confident in our findings. Treatments to be examined in 2013 include the timing and number of spray-inoculations with and without the addition of carborundum, which has demonstrated an ability to aid infection by the bacterium. Assessment scales used to evaluate BLS in the field and greenhouse will be compared.
2) **Establish if BLS is of economic importance.** We anticipated that we would wrap up this objective in 2012 however the 2012 season was challenging and we obtained useful data only from the UMN experiment at St Paul. Thus we proposed to repeat the work in 2013 to confirm the results, anticipating publishable data from the 2013 field season.
   - UMN: undertook field experiments in 2013 in St. Paul and Crookston on a limited number of wheat cultivars including; ND495 (R), Blade (R), RB07 (MS) and Knudson (S), to establish yield losses to at least three levels of severity of BLS. Disease development was assessed and the trial was harvested for yield. This experiment aimed to document the impact of BLS on wheat yield and quality and to confirm the data generated in 2011 and 2012.
   - SDSU repeated a field experiment conducted in 2010 and 2011 to examine the yield and quality reduction in spring wheat following challenge with the BLS pathogen. Multiple genotypes representing three distinct levels of resistance were used. Resistant genotypes include SD4205 and SD4148, Russ has a medium level of resistance, and Select and SD4011 were included as susceptible genotypes.
3) **Examine the structure of the pathogen population.** We know that there is considerable genetic variability in this pathogen, however we need to determine if this is of biological importance to the disease. The host ranges of *X. translucens* pathovars overlap. Although *X.t.* pv. *undulosa* is defined as infecting wheat, *X.t.* pvs. *translucens*, *secalis*, *cerealis, poae, phlei* and *graminis* have all been demonstrated to be pathogenic on wheat. By differentiating between the pathovars present in our region, breeding efforts can be more accurately tailored to the range of pathovars causing disease. Determining if there are 'races' is also critical to understanding if the pathogen is likely to change in response to disease control measures and in establishing a representative population for use in screening germplasm.
   - UMN: will continue to explore various fingerprinting techniques that will lead to the elucidation of the pathovar classification and detection of ‘races’ within the pathogen population. MLST (Multilocus Sequence Typing) analysis is in progress to generate genetic fingerprints for a collection of pathogen isolates, including isolates from SDSU. Sequence data has been generated for a subsample of UMN’s isolate collection. In March 2013, Rebecca Curland (UMN Research Fellow) travelled to Dr. Carolee Bull’s laboratory in Salinas, CA to receive training in multilocus sequence analysis.
   - UMN: Isolates from unique genetic groups were screened in the greenhouse to develop phenotypic profiles for isolates of interest.
4) **Develop a cooperative regional BLS screening nursery.** This nursery screened commercially available wheat cultivars (public and private releases) and advanced breeding lines to provide information on relative variety performance to growers. We also supported the breeding programs within each state by screening additional breeding lines and parental lines of populations developed to identify genetic resistance to BLS. Information from these screenings are still being collated but will be provided to the breeding programs to provide valuable information on the reaction of their wheat germplasm to BLS. Producers are currently able to access information on the reaction of wheat cultivars to BLS through the MN Variety Trials Bulletin. Thus far neither SD nor ND has sufficient confidence to present the reaction of wheat cultivars to BLS in their state publications.
   - UMN, SDSU, NDSU: inoculated nurseries were established in each state to screen a cooperative nursery encompassing entries from the UMN, SDSU, NDSU, Limagrain, WestBred and Agripro spring wheat breeding programs. Entries were selected in consultation with all the breeders whose

material is represented.

- UMN: screened the 2013 Advanced Yield Trial (AY1) as well as several populations provided by Jim Anderson that are believed to be segregating for their response to BLS.
- SDSU: screened the AYT and PYT consisting of approximately 110 breeding lines in total. SDSU also conducted family-based mapping on a set of wheat lines currently in development. Using this approach we hoped to identify QTL's associated with the resistance to bacterial leaf streak. To evaluate lines in the mapping study, the double-digit scale will be utilized to score the entire plot in addition to scoring of flag leaves among ten random plants per line.
- NDSU: established a field nursery for BLS screening in Fargo and initially screened approximately 100 wheat genotypes including current and newly released cultivars as well as breeding lines from spring wheat breeding programs in this region. The disease inoculation and assessment followed protocols developed in MN.

5) **Examine the influence of foliar fungicides on BLS development.** While it has been speculated that the increase in BLS observed in recent years may be related to an increased use of fungicides in wheat production, the interaction of BLS and other foliar diseases and fungicide applications has not been examined. Under this objective we proposed to determine the impact - either positive or negative - of fungal pathogens and/or fungicides on BLS development.
   - UMN: undertook a field experiment, collaboratively with Limagrain, in 2013 to examine the development of BLS on wheat lines. These experiments included four treatments: inoculated (or not) with *X. translucens* (BLS) and treated (or not) with Prosaro. Disease development of BLS was assessed and the trial was harvested for yield. This experiment repeated a study conducted at Foxhome in 2012 that provided preliminary data on the impact of fungicides on BLS development.
   - SDSU: undertook a field experiment in 2013 on two wheat lines to examine the development of BLS in plots. The experiments included treatments: inoculated with *X. translucens* (BLS), inoculated with *X. translucens* and *P. tritici-repentis* (Tan Spot) and inoculated with *X. translucens* and treated with a fungicide (Prosaro). All inoculation and fungicide treatments were applied within 48 h of each other. Disease development of BLS, tan spot and any other diseases were assessed and the trial was harvested for yield. This experiment has begun to document the impact of fungal pathogens and/or fungicide applications on BLS development.

6) **Strengthen our collaborative research effort on BLS.** With new Plant Pathology faculty at both SDSU and NDSU face-to-face meetings of the researchers involved in this project was necessary both to capitalize on the gains we have made in developing techniques to work with BLS and to develop a plan to transition to research focused on disease control.

<u>Economic Benefit to a Typical 500 Acre Wheat Enterprise:</u>

We have demonstrated that Bacterial Leaf Streak is of economic importance to the wheat industry. Data has been generated that growers can use to select wheat varieties that are less susceptible to BLS. Techniques have been established that allow us to screen breeding materials and therefore provided the tools to make progress in disease resistance introgression.

10: RELATED RESEARCH

This is a regional collaborative project involving pathologists in three states. We have established close relationships with extension personnel and wheat breeding programs in each state that benefited the project in 2013 by providing field observations of the distribution of BLS, collection of symptomatic plants for isolate collection, wheat germplasm and advice.

With funding from ND wheat Commission, NDSU is undertaking disease evaluation of a large number of wheat materials in the greenhouse. These materials include triticales, emmer wheat, synthetic wheat, and wheat-alien species derivatives. NDSU is also taking on an effort to map the partial resistance to BLS that exists in some wheat genotypes using currently available populations.

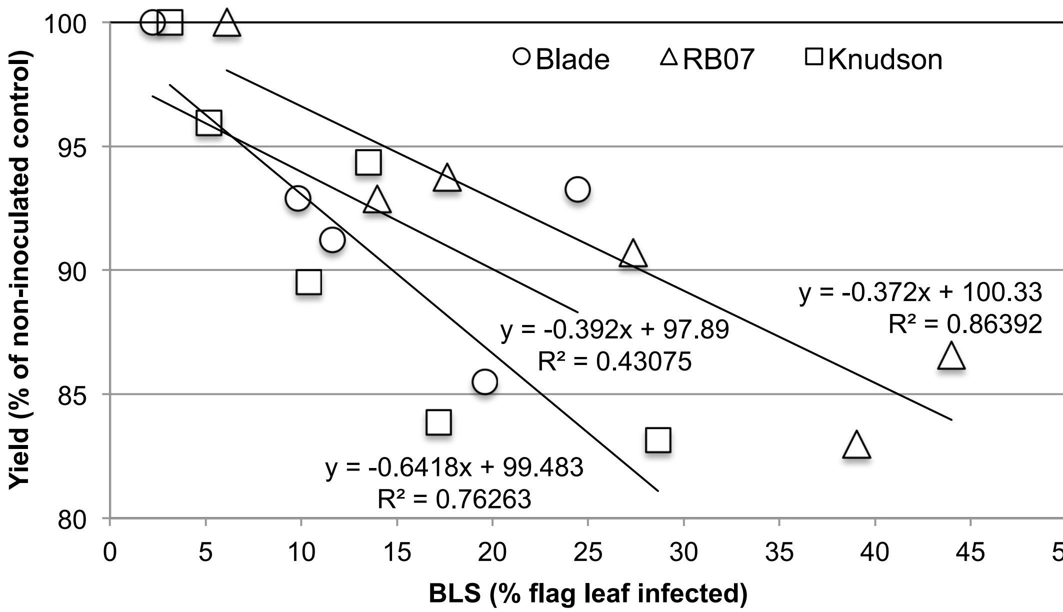
11: RECOMMENDED FUTURE RESEARCH

Although much progress has been made - we have refined and developed reliable screening methods both in the field and in the greenhouse, have embarked on a collaborative screening nursery and have started screening breeding materials in the greenhouse and field in each state there is still progress to be made working with BLS. Additional research is needed to develop simple and easy-to-use evaluation protocols in the greenhouse that can be applied to screen large numbers of wheat lines.

In 2013 we undertook our first experiments to study the effects of foliar fungicides on the development of BLS. The preliminary data has shown BLS severity was affected by the fungicide application, but the experiments have been repeated before we can draw any solid conclusion.

From our collaborative screening effort in 2013 it was found that the majority of current cultivars and many advanced lines from the regional breeding programs are susceptible to BLS. We plan to move forward using regional collaborative nursery to screen wheat materials from diverse sources for their response to BLS. The primary goal of this effort will be to identify genotypes with high levels of resistance that can be provided to breeding programs for developing BLS resistant cultivars or germplasm.

Previous studies suggest that resistance to BLS is governed by multiple genes and quantitatively inherited; in addition, the evaluation of plant responses to BLS is challenging and influenced by environmental conditions; therefore, we believe that there is a need to develop DNA markers to facilitate the use of marker assisted selection for introgressing resistance this disease in wheat.



Appendix 1: Effect of BLS infection on yield for each of three hard red spring wheat cultivars (Blade, RB07 and Knudson) (St Paul, 2013)

Appendix 2: Response of named wheat varieties to BLS infection. Data from the 2013 cooperative nursery planted at four locations (St Paul, MN; Crookston, MN; Fargo, ND and Brookings SD).

Variety	BLSCN#	MN-StP	MN-Crk	ND	SD	Mean
Blade	114	4.75	5.00	6.25	2.75	4.69
LCS Albany	95	4.25	5.00	6.25	3.50	4.75
Hattrick	44	4.25	6.25	5.25	4.00	4.94
Faller	10	5.25	4.75	6.50	4.00	5.13
Breaker	31	5.50	5.50	5.50	4.25	5.19
Cromwell	61	4.00	6.50	7.00	3.75	5.31
Vantage	86	5.25	6.25	6.50	3.50	5.38
Knudson	57	5.00	6.00	7.25	3.50	5.44
Advance	113	4.75	6.00	7.00	4.25	5.50
Sabin	102	4.75	5.75	6.00	5.75	5.56
Alpine	110	4.50	5.00	7.75	5.00	5.56
WB-Gunnison	35	5.25	6.25	6.50	4.50	5.63
Jenna	13	5.00	7.75	6.25	4.50	5.88
WB9879CLP	74	6.50	6.75	6.00	4.25	5.88
SY Rowyn	89	6.00	5.00	8.00	4.75	5.94
Norden	5	5.50	7.00	7.75	4.00	6.06
LCS Powerplay	30	4.75	4.75	8.75	6.00	6.06
Granite	105	5.00	6.00	7.25	6.00	6.06
LCS Breakaway	28	5.50	5.25	8.00	5.75	6.13
Glenn	94	5.75	6.75	7.25	4.75	6.13
Edge	43	5.75	6.75	7.50	4.75	6.19
WB-Digger	107	5.25	6.25	8.00	5.25	6.19
Barlow	18	4.75	6.50	7.75	6.00	6.25
Elgin-ND	111	5.25	7.25	8.25	4.25	6.25
Rollag	106	5.25	7.50	7.25	5.25	6.31
Forefront	92	5.50	7.00	7.50	5.50	6.38
Linkert	66	5.25	7.00	8.25	5.25	6.44
SY605 CL	100	5.50	6.75	8.25	5.25	6.44
SY Soren	46	6.50	6.50	8.00	5.00	6.50
Select	54	4.75	6.25	8.50	6.50	6.50
RBO7	16	6.25	7.75	7.25	5.50	6.69
SY Tyra	67	6.00	7.75	7.75	5.25	6.69
Samson	90	6.50	8.25	7.00	5.25	6.75
Brennan	71	6.50	7.50	8.75	5.25	7.00
WB-Mayville	17	6.75	7.50	8.25	6.25	7.19

Within columns, the standard deviation (S.D.) of the treatment (variety) mean from the location mean are color coded as follows:

-3 S.D. or more
-2 S.D.
-1 S.D.
+1 S.D.
+2 S.D.
+3 S.D. or more

Appendix 3: Effect of fungicide and tan spot (Ptr) infection on the development of BLS (Xt) in wheat cultivars Briggs and Select (Brookings, 2013)

	BLS (1-9 scale)	
Treatment	Briggs	Select
1 Xt only	5.7	7.7
2 Xt + Ptr	4.7	6.3
3 Xt + Ptr +Prosaro	4.0	6.0
4 Ptr only	4.3	5.0
5 Non-inoculated check	2.0	2.7