# Collaborative Research on Wheat Diseases: Bacterial Leaf Streak, Root and Crown Rots and Viral Diseases of Wheat

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## Research Question

This project continued our research effort addressing emerging diseases of wheat that are responsible for yield and quality losses in the Upper Great Plains, including Minnesota. The project focused on three diseases; bacterial leaf streak (BLS); root and crown rots; and viral diseases.

Bacterial leaf streak of wheat, caused by *Xanthomonas translucens* pv. *undulosa*, is prevalent in Minnesota and is currently considered to be the second most important disease of wheat in the state, after Fusarium head blight (FHB). Managing BLS is difficult due to the lack of highly resistant cultivars and other effective tools, especially as fungicides are ineffective against bacteria. Our research aimed to improve our understanding of the disease and to develop methods for disease control. Our specific objectives related to BLS included:

1.1. Co-ordinate the BSL cooperative nursery, testing commercial cultivars from regional wheat breeding programs
1.2. Identify sources of resistance to BLS using field and greenhouse screens
1.3. Undertake genetic analysis of resistance to BLS in identified sources of resistance
1.4. Examine the role of common weeds and crop residues in the epidemiology of BLS
1.5. Examine genetic variation within pathogen populations
1.6. Disseminate information to wheat growers

The root and crown diseases of wheat may cause significant yield losses, although they frequently go unnoticed. Root diseases generally compromise the root system, affecting the ability of the plant to take up water and nutrients, thus root diseases are especially damaging in years when water is limiting during grain filling. Surveys from 2012 to 2015 identified several root rot pathogens impacting wheat crops in Minnesota. In this project we have continued research to identify the pathogens causing root disease and to understand the response of the wheat germplasm to these pathogens. Specific objectives on wheat and crown diseases included:

2.1. Validating and further developing screening methods for reaction to root rot pathogens
2.2. Screening commercial cultivars and advanced breeding lines for resistance to Fusarium crown rot (FCR)
2.3. Identify sources of resistance to FCR under field and greenhouse conditions
2.4. Disseminate information to wheat growers

Viral diseases such as barley yellow dwarf, caused by barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CTDV); and wheat streak, caused by wheat streak mosaic virus (WSMV), have been severe in wheat in years where conditions are favorable to the insect and mite vectors that transmit these viruses. In this project we aimed to identify the viral threats to wheat production in the region and characterize the viruses identified. We also aimed to provide recommendations on the reaction of commercial varieties and to direct breeding efforts with respect to virus diseases. Specific objectives on virus diseases included:

3.1. Validate diagnostic tests that characterize the viruses found in association with wheat
3.2. Examine the distribution of cereal viruses in spring and winter wheat
3.3. Determine the occurrence and distribution of cereal viruses on non-wheat hosts
3.4. Develop management strategies for viral diseases
3.5. Disseminate information to wheat growers

## Results

*Bacterial Leaf Streak:*
In 2016 we tested released varieties and advanced lines in a regional cooperative nursery (BLSCN). The 120 entries came from seven wheat breeding programs (3 public and 4 private) in the Upper Great Plains. The BLSCN was established at four locations (St Paul, Crookston, Fargo, ND and Brookings, SD). The data from all four locations indicate that significant differences were observed in these materials for their reaction to BLS under field conditions (See Table provided in the Appendix). The information obtained on the response of released varieties and elite germplasm will be utilized by regional wheat breeding programs to the benefit of growers. Information on the response of released germplasm to BLS collected in 2016 will be combined with previous data sets and the overall evaluations will be disseminated to Minnesota growers through the MN variety trials bulletin.

In 2016 we conducted surveys looking for BLS symptoms in wild grasses (wild oats, foxtail, barnyard grass and others) and in other grass hosts including intermediate wheatgrass, oats and wild rice in Minnesota. We made collections of symptomatic tissues and are currently working to isolate and identify the bacteria associated with these host plants. Simultaneously we are working to establish greenhouse protocols to establish the host range of these isolates and thus establish if these are relevant to the *Xanthomonas* population inciting BLS on wheat.

We have characterized our entire isolate collection (including isolates from MN, ND and SD) with MLSA (multilocus sequence analysis). MLSA revealed that both *Xanthomonas translucens* pv. *undulosa* (pathogenic on wheat and barley) and *X. translucens* pv. *translucens* (pathogenic on barley) are present in the region. Overall, isolates from wheat appear to be pathovar *undulosa*, though a couple isolates from wheat grouped with *translucens* isolates which suggests that host specificity may not be clearly defined. Isolates in the *undulosa* group are less genetically diverse (monophyletic) compared to isolates within the *translucens* group (3 distinct clades). We intensively sampled two wheat fields that were naturally infected with BLS and obtained isolate collections (25 per field) that represent populations within a field site. MLSA of these isolates is in progress and results will be reported in future updates.

*Root and Crown Diseases:*
Root rot disease work continued in 2016. We completed the isolation of fungal pathogens from the samples collected and have identified *Fusarium* species using morphological techniques. We are working toward developing protocols to confirm the identity of the isolated fungi using molecular and/or DNA sequencing. The results from the surveys conducted thus far (2012 onward) have determined that the prevalence of species of *Fusarium*, particularly *F. graminearum* has increased compared to previous surveys. This has helped us prioritize research needs and has provided the isolates needed for establishing screenings for host resistance to root and crown rots. In 2016 we tested a protocol for screening materials in the field that, while effective in generating disease, was not suitable for experiments of the scale needed to effectively and reliably screen germplasm. Until we are able to establish a reliable inoculation protocol our ability to screen germplasm for resistance remains limited. Our preliminary findings indicate that resistance to Fusarium head blight (FHB or scab) is limited and independent of resistance to crown and root resistance, as has been reported in the scientific literature.

*Virus Diseases:*
Efforts in 2016 focused on validating diagnostic tests that characterize the viruses found in association with wheat. Collections of barley yellow dwarf symptomatic material from 2013, 2014 and 2015 were used to refine the molecular diagnostic tools for Barley yellow dwarf virus (BYDV) and Cereal yellow dwarf virus (CYDV), the causes of barley yellow dwarf. Molecular diagnostic tools are important because they represent the most efficient and sensitive methods for pathogen detection, as opposed to immunoassays such as ELISA (enzyme-linked immunosorbent assay). In addition, there at least five different strains of BYD/CYDV, not all of which can be detected using ELISA. During the course of 2015 work, it became apparent that published protocols for molecular diagnostics of BYDV (Malmstrom and Shu, 2004) needed to be refined because these gave false negatives in some of our samples. Various methods were tested and a modified protocol established that now appears to work consistently for diagnostic purposes in our region. In the spring of 2016, this methodology was also adapted into a protocol for testing cereal aphids, in both bulked (many aphids) and individual (single aphid) samples. Testing winged aphid populations when they arrived in the southern part of Minnesota enabled estimates to be made of the proportion of the vector population carrying BYDV/CYDV.

Refinements of the diagnostic assay has taken much longer than anticipated, but was crucial to the objectives of this work. We now have an assay for making greater progress on Objectives 3.2 and 3.3.

Results from the samples we collected, along with those collected by our NDSU and SDSU colleagues, indicate that the predominate strain of BYDV/CYDV in hard red spring wheat is BYDV-PAV. This finding in in accordance with data from surveys conducted over 10 years ago in the region. However, in other host species such as oat, BYDV-PAV was found less frequently and other strains such CYDV-RMV or CYDV-RPV seemed to be more common. More extensive sampling of other host species will be required to determine if this finding is born out with a larger sample size and broader geographic context.

## Application/Use

Our ability to determine the prevalence and impact of the diseases of wheat is essential to develop disease control strategies, including resistance. Developing resistant wheat germplasm will rely on collections of pathogens and the development of effective screening methods, both to identify sources of resistance and to introgress the resistance into adapted germplasm. In 2016 we have both utilized protocols we have developed to establish screening nurseries for BLS and have continued work to improve screening protocols for BLS and the root diseases. Similarly, we have worked toward identifying the root rot fungi and viruses present in wheat.

## Materials and Methods

Bacterial leaf streak: By the start of this project we had developed the basic protocols needed to work with BLS and developed a regional cooperative nursery (BLSCN) in which released cultivars and advanced lines from all wheat breeding programs (public and private) in the Upper Great Plains are being screened annually for resistance to BLS. Screening nurseries were also used to identify additional sources of resistance. Annual field screening nurseries are critical to the ultimate goals of the research - host resistance -and this work is being done cooperatively with Drs Shaukat Ali (South Dakota State University) and Zhaohui Liu (North Dakota State University).

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We examined populations of *Xanthomonas* in wheat for their host preference so that we can use this information to inform which isolates are selected for use in germplasm screening. To evaluate the contribution of weeds and crop residues to reservoirs of the BLS pathogen, collections of *Xanthomonas* were obtained from crop residues and common weed species, including wild rice throughout Minnesota. The host preference and genotype of each isolate was determined using molecular tools, including multi-locus sequence typing (MLST), a technique which was established in a previous project. Host range inoculation studies are being undertaken in the greenhouse to complement this work.

Wheat Root and Crown Diseases: Over the last five years we have developed a better understanding of the root and crown rots in wheat. Field surveys, conducted collaboratively across the three states, have examined the distribution and prevalence of root rot pathogens.

Fungal pathogens from the 2012-2016 surveys have largely been identified using morphological means and added to our collection. Efforts to identifying the fungi by DNA sequencing have continued. We have established work in the laboratory, greenhouse and field to test methods for inoculating the roots and stem bases of wheat plants with *Fusarium* spp. These efforts continued in 2016 and should ultimately facilitate our ability to screen breeding materials for reaction to the prevalent root rot pathogens in the region. In 2016 a field nursery was established in St Paul to test the most promising inoculation protocols developed in the laboratory.

Virus Diseases: The data collected over the last three years has given us information on the strains prevalent in commercial crops around the state. Molecular methods for the identification of strains were used in strain identification. Surveys were conducted around the state in 2016 by sampling grasses in ditches next to fields with symptoms of BYDV. The molecular identification methods are being utilized to determine BYDV present in collected samples. Samples were also collected in South Dakota by Dr. Byamukama (South Dakota State University). The 2016 samples are also being evaluated for the presence of wheat streak mosaic virus in collaboration with Dr. Byamukama.

## Economic Benefit to a Typical 500 Acre Wheat Enterprise

We have demonstrated that bacterial leaf streak (BLS) is of economic importance to the wheat industry and data has been generated that a grower can use to select wheat varieties that are less susceptible to BLS. The data gathered from this project demonstrate that root rot and viral diseases are prevalent in commercial wheat fields in Minnesota. It appears that two root rot pathogens, *Fusarium* and *Bipolaris*, are abundant and that they likely contribute significantly to yield losses, particularly in years when moisture is limiting in the latter part of the growing season. Similarly BYDV appears widespread in wheat and is likely impacting yields in some years. While this information does not provide any immediate benefit to the grower an awareness of the problem is a first step to the control of the root rots and viral diseases of wheat.

## Related Research

This is a regional collaborative project involving pathologists in three states. We have established close relationships with research and extension plant pathologists and the wheat breeding programs (public and private) in each state. The regional wheat breeding programs have benefited the project by providing field observations of the distribution of diseases, collection of symptomatic plants for isolate collection and wheat germplasm. The wheat breeding programs in the region (public and private) have benefitted from knowledge of the reaction of released and advanced breeding lines to BLS.

## Recommended Future Research

*Bacterial leaf streak:* Although we continue to make progress with BLS, additional research is needed. Our collaborative screening efforts have determined that the majority of our wheat cultivars and many advanced lines from the regional breeding programs are at least moderately susceptible to BLS and that there is generally less resistance in the regionally available germplasm than is desirable. We plan to continue using screening nurseries to test wheat lines for their response to BLS and plan in 2017 to expand the materials we are examining in the hopes of identifying additional and improved sources of resistance. 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. We anticipate continuing our studies examining the pathogen population, to determine the host range of the *X. translucens* pv. *undulosa* pathovars associated with BLS of wheat and other grasses.

*Root Rots:* The survey of root diseases we have already conducted have demonstrated that root rot pathogens are readily found in wheat crops in Minnesota and that they most likely have a significant negative impact on yield. We have made progress, albeit rather slow, in developing testing methods suitable for inoculating plants with Fusarium spp. in the field and greenhouse. As was anticipated from the start from this project, working with these root diseases has proven challenging. We plan to continue work in 2017 with a focus on developing a greenhouse-based screen suitable for high throughput phenotyping of germplasm.

*Viruses:* Efforts concerning BYDV and CYDV will continue in 2017. We plan to determine the role of grass species, other than crops, which are hosts for BYDV/CYDV using

the improved molecular diagnostic techniques we have developed. This work will allow us to determine if the grasses growing in ditched and roadsides and other areas adjacent to commercial wheat crops are of concern in the epidemiology (survival and spread) of the disease in Minnesota. We also plan to try to develop a quantitative PCR method suitable for the detection and quantification of BYDV/CYDV now that we have a consistent assay for molecular detection. These efforts will be both informative to resistance breeding efforts and disease risk assessment.

## Publications


Winter, M., Samuels P.L., Dong, Y and Dill-Macky. (2016). Deoxynivalenol (DON) and nivalenol (NIV) play a role as virulence factors for wheat root and stem base infection by *Fusarium culmorum* and *F. graminearum*. *Abstract In Proceedings of the 2016 National Fusarium Head Blight Forum*, St. Louis, Missouri, USA, December 4-6, 2016.

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Smith, M.J., Dill-Macky, R., Curland, R., Ishimaru, C.I, and Wiersma, J. (2015) Bacterial Leaf Streak and Black Chaff of Small Grains. University of Minnesota Extension Publication.


## Appendix

Mean BLS score (1-9 scale, where 9 is the highest level of disease) for named cultivars in the 2016 BLS cooperative nurseries established at four locations in 2016, along with the four-location mean. Values provided are the means of four replicates. The colors; dark green, light green and yellow, indicate where the values provided are 3, 2 and 1 standard deviations below the location mean, respectively, while the colors orange, red and maroon indicate that the values are 1, 2 and 3 standard deviations above the location mean, respectively.

| Variety | St. Paul, MN | Crookston, MN | Fargo, ND | Brookings, SD | 4 Loc. Mean |
|---|---|---|---|---|---|
| Cromwell | 3.50 | 2.75 | 5.25 | 4.25 | 3.94 |
| LCS Trigger | 3.50 | 2.75 | 6.25 | 3.50 | 4.00 |
| Boost | 3.75 | 3.50 | 5.00 | 4.00 | 4.06 |
| Blade | 4.75 | 2.50 | 5.75 | 3.50 | 4.13 |
| Prosper | 3.75 | 3.25 | 6.50 | 3.75 | 4.31 |
| Prevail | 4.25 | 3.00 | 5.75 | 4.50 | 4.38 |
| LCS Hattrick | 3.75 | 3.75 | 6.50 | 4.25 | 4.56 |
| Advance | 4.25 | 3.75 | 6.25 | 4.25 | 4.63 |
| Shelly | 3.75 | 3.50 | 8.00 | 3.75 | 4.75 |
| Surpass | 4.75 | 3.25 | 6.75 | 4.50 | 4.81 |
| LCS Nitro | 4.00 | 4.50 | 7.00 | 4.00 | 4.88 |
| Forefront | 4.75 | 4.25 | 6.50 | 4.25 | 4.94 |
| LCS Iguaca | 4.00 | 3.75 | 8.00 | 4.00 | 4.94 |
| Barlow | 5.25 | 3.00 | 7.00 | 4.75 | 5.00 |
| Rollag | 4.75 | 3.00 | 7.75 | 4.50 | 5.00 |
| Bolles | 3.50 | 5.00 | 7.25 | 5.00 | 5.19 |
| Focus | 5.00 | 4.00 | 8.00 | 4.25 | 5.31 |
| Knudson | 5.50 | 4.75 | 6.50 | 4.50 | 5.31 |
| Glenn | 5.00 | 4.00 | 7.25 | 5.25 | 5.38 |
| LCS Prime | 5.25 | 5.00 | 7.50 | 4.75 | 5.63 |
| LCS Pro | 5.00 | 4.50 | 8.00 | 5.00 | 5.63 |
| Linkert | 5.50 | 4.25 | 8.00 | 4.75 | 5.63 |
| Norden | 6.00 | 3.50 | 8.25 | 4.75 | 5.63 |
| RB07 | 5.25 | 5.00 | 6.75 | 6.25 | 5.81 |
| LCS Breakaway | 6.25 | 3.75 | 7.75 | 5.75 | 5.88 |
| LCS Powerplay | 5.50 | 5.00 | 8.00 | 5.00 | 5.88 |
| Samson | 5.50 | 4.50 | 7.75 | 5.75 | 5.88 |
| LCS Anchor | 5.50 | 4.00 | 8.75 | 6.00 | 6.06 |
| Select | 6.50 | 6.00 | 8.00 | 6.25 | 6.69 |