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

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Research Questions

This project aimed to address diseases that impact the yield and quality of wheat in the Upper Great Plains, especially Minnesota, that are not otherwise being adequately addressed by research efforts in the region. The project has focused on three diseases; Bacterial Leaf Streak (BLS); Root and Crown Rots; and Viral Diseases that have been of increasing concern to wheat production in Minnesota. The ultimate goal of the project is to deliver effective disease control measures for all of these diseases.

Bacterial leaf streak of wheat, caused by *Xanthomonas translucens* pathovar (pv.) *undulosa*, is prevalent in Minnesota and is the second most important disease to impact wheat productivity in the state, after Fusarium head blight (FHB). Managing BLS is difficult due to the lack of highly resistant cultivars and other best practices, as fungicides are ineffective against bacteria. Our research has aimed to improve our understanding of the pathogen 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 all wheat breeding programs in the region
1.2. Identify sources of resistance to BLS using field and greenhouse screens
1.3. Conduct studies to examine the epidemiology of BLS to determine the host range of the pathogen
1.4. Examine variation in pathogen populations
1.5. Disseminate information to wheat growers

The root and crown diseases of wheat may cause significant yield losses, although they frequently go unnoticed. But growers. Root diseases generally compromise the root system, affecting the ability of the plant to take up water and nutrients. Root diseases are especially damaging to yields in years when water is limiting during the grain filling period. We have made significant progress in developing methods for screening plants against the *Fusarium* spp. that incite root rots in the greenhouse. These greenhouse screens have allowed us to identify sources of resistance and start screening breeding materials for reaction to Fusarium crown rot. Specific objectives on wheat and crown diseases included:
2.1. Validate the greenhouse methods for determining the reaction to crown rot in the greenhouse
2.2. Screen commercial cultivars and advanced breeding lines for resistance to Fusarium crown rot
2.3. Screen a wheat population that may be segregating for response to crown rot to identify resistant lines
2.4. Identify sources of resistance to Fusarium crown rot
2.5. 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. This project has aimed to provide additional information on the strains prevalent in commercial wheat crops along with the grass species found adjacent to small grains fields. We also examined the reaction of commercial varieties to virus diseases. Specific objectives on virus diseases included:
3.1. Examine the distribution of cereal viruses in spring and winter wheat
3.2. Determine the occurrence and distribution of cereal viruses on non-wheat hosts
3.3. Develop management strategies for viral diseases
3.4. Disseminate information to wheat growers

Results

Bacterial Leaf Streak: In 2018 we tested released varieties and advanced lines in a regional cooperative nursery (BLSCN). The 106 entries came from six wheat breeding programs (3 public [UMN, NDSU, SDSU] and 3 private [BASF, Bayer CropScience, Syngenta) 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. The information obtained on the response of released varieties and elite germplasm has been provided to the regional wheat breeding programs to the benefit of growers. Information on the response of released germplasm to BLS collected in the 2018 BPSCN will be combined with previous data sets and the overall evaluations will be disseminated to Minnesota growers through the MN variety trials bulletin and other publications.

In 2018 we continued our work examining the role that wild grasses and other grass hosts play in the epidemiology of BLS in Minnesota. We utilized a collection of *Xanthomonas translucens* isolates collected from the weed hosts to conduct a molecular analysis of the diversity of the pathogen. Sequence data from four loci (*rpoD, dnaK, fyuA, gyrB*) was generated for 105 *Xanthomonas*

translucens isolates originating from quackgrass, wild oat, foxtail barley, perennial ryegrass, green foxtail, smooth bromegrass, cultivated wild rice, intermediate wheatgrass, barley, and wheat. A phylogeny of these isolates was constructed using multi-locus sequence analysis (MLSA) and used in corroboration with greenhouse seedling assays to identify the isolates to pathovar level. All isolates originating on cultivated wild rice, intermediate wheatgrass, wheat, and weedy grasses, except smooth bromegrass, were identified as *X. translucens* pv. *undulosa*. All isolates originating on smooth bromegrass were identified as *X. translucens* pv. *cerealis* and all isolates originating on barley were identified as *X. translucens* pv. *translucens*. Each isolate was also assigned a sequence type number based on the sequence data generated for the four housekeeping genes to further evaluate diversity. The results of this work suggest that there are several distinct subpopulations of the pathogen and that only some of the grass weed species examined serve as alternative hosts for the BLS pathogen populations that infects wheat.

In 2018 select isolates of *X. translucens* collected from wild oat, quackgrass, foxtail barley, wheat, and barley were screened for the second year in the field on three hosts; wheat, barley, and oats. Disease was assessed as severity on a 0-9 scale and vertical disease progression on a 0-9 scale. The isolates examined incited disease on both wheat and barley, but not oat. The statistical analysis of this data will be completed over the winter months.

Isolates representing unique types were used to validate the LAMP assay using primers designed by Langlois et al. (2017) to identify *X. translucens* pathovars. Results confirmed that primer sets used to detect all pathovars of *X. translucens* were valid for all isolates tested. Primer sets used to detect pathovars which cause BLS in small grains (pvs. *undulosa, translucens, secalis*) were valid for all isolates tested, except *X. translucens* pv. *translucens* isolates with sequence type 24. The validation of this test (the LAMP assay) will enable us to more rapidly identify if strains of *X. translucens* are indeed pathogens of wheat.

Root and Crown Diseases:
In the 2017/18 fall and spring seasons we examined a wheat populations that appear to be segregating for reaction to crown rot in greenhouse experiments. We are still in the process of re-screening the material to confirm our preliminary data and should complete the screening this population in late 2018. The ultimate goal of this work is to identify progeny with improved resistance that can serve as adapted donors of resistance in the hard red spring wheat breeding programs in the Upper Great Plains.

Virus Diseases:
During the 2017 (Fig.1A) and 2018 (Fig. 1B) growing seasons 519 and 708 grass samples from 193 and 241 locations were collected, respectively. Grasses were sampled from field margins and ditches adjacent to commercial small grain fields. Thirty species of grass were identified (with one unidentified grass). The most prevalent species were *Bromus inermis* and *Poa pratensis* accounting for 24 and 26% of samples collected, respectively.

Whole plant RNA was extracted from plants and cDNA synthesis conducted. Polymerase chain reaction (PCR) specific for BYDV detection was conducted modified from the multiplex PCR methodology of Malmstrom and Shu (2004) in three singleplex reactions. So far, 203 samples have been processed for initial detection of BYDV. Two samples from *Elymus repens* (Quakgrass) from the same location in MN have been positive for the presence of BYDV. These will be further tested to elucidate the strain of BYDV present. Work continues in the lab to finishing processing the samples through RNA extraction, cDNA synthesis and PCR. By the end of 2018 we expect to have completed the strain identification and will be able to determine the distribution of these strains around the state. Based on this information we will be able to make recommendations and disseminate information to growers in best management practices.

Application/Use

Developing effective and durable resistant germplasm to the diseases of economic importance to wheat in Minnesota relies in the development of effective screening methods to identify sources of resistance and to introgress the resistance into adapted germplasm, along with an understanding of the epidemiology and biology of the pathogens.

In 2018 we have continued our screening efforts in field nurseries for BLS and greenhouse screening for crown rot of wheat. We have also made significant progress in understanding the diversity of the pathogen populations that inform future breeding efforts and the development of other disease control practices.

Material and Methods

Bacterial leaf streak: We have established a cooperative regional nursery (BLSCN) in which released cultivars and advanced lines from 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 Dr Shaukat Ali (South Dakota State University) and Dr Zhaohui Liu (North Dakota State University).

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 multilocus sequence typing (MLST), a technique which was established in a previous project. Host range inoculation studies were 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, examined the distribution and prevalence of the root rot pathogens. We have established laboratory and greenhouse methods for inoculating the roots and stem bases of wheat plants with *Fusarium* spp. These efforts continued in 2018 and have ultimately facilitated our ability to screen breeding materials for reaction to the prevalent root rot pathogens in the region.

Virus Diseases: The data collected in previous surveys has given us information on the strains prevalent in commercial crops around the state. PCR detection of BYDV strains, has identified the grasses infected with BYDV and indicated where these strains are similar to those present in wheat.

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 rots 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. Information on the prevalence of these diseases is of immediate benefit to the grower by increasing an awareness of disease problems impacting wheat production. The development and introgression of host resistance provides economic and environmentally sustainable control of wheat diseases.

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 Minnesota and with our neighboring states. 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 especially benefitted from information on the reaction of released and advanced breeding lines to BLS.

Recommended Future Research

Bacterial leaf streak: Our collaborative screening efforts have provided robust data on the reaction of commercial what cultivars to BLS. The majority of our wheat cultivars, and many advanced lines from the regional breeding programs, are at least moderately susceptible to BLS thus additional efforts to identify source of resistance are warranted. We plan to continue using screening nurseries to test wheat lines for their response to BLS and have expanded the materials we are examining in order to identifying additional and improved sources of resistance. BLS resistance appears to be governed by multiple genes and quantitatively inherited. In 2019 we plan to undertake a collaborative effort with Dr Zhaohui Lui (NDSU) to verify the presence of a QTL for BLS resistance that has been tentatively identified in a synthetic wheat population he has been working with. We anticipate completing our studies examining the pathogen population to determine the host range of the *X. translucens* pv. *undulosa* pathovars associated with BLS of wheat, other crops and grassy weeds. Given that it appears that the host range of the pathogen is considerable broader than wheat alone, an understanding of the role that the grassy weeds and alternative host crops play in the epidemiology of this disease may direct research to specific disease control options for growers.

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 good progress 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, the root diseases have proven challenging host-pathogen interactions to understand and manipulate. We have continued to make steady progress in our understanding of the root rots in 2018 having continued our efforts in developing greenhouse protocols that are of value to breeding efforts. We plan to continue this work in 2019.

Publications

Journal Articles

Winter, M., Samuels, P.L., Dong, Y., and Dill-Macky, R. (2018). Trichothecene production is detrimental to early root colonisation by *Fusarium culmorum* and *F. graminearum* in Fusarium crown and root rot of wheat. *Plant Pathology*, Accepted for publication. [Posted online on 16 Aug 2018, doi: 10.1111/ppa.12929]

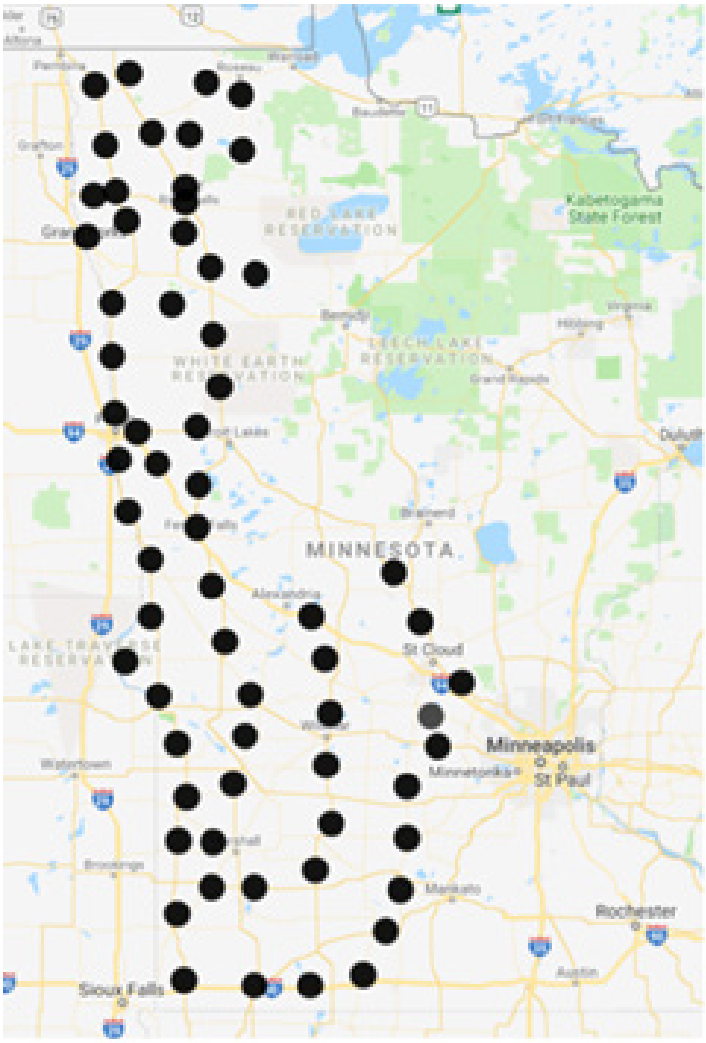

Fig. 1A: distribution of sampling locations for fall 2017 indicated by black dots

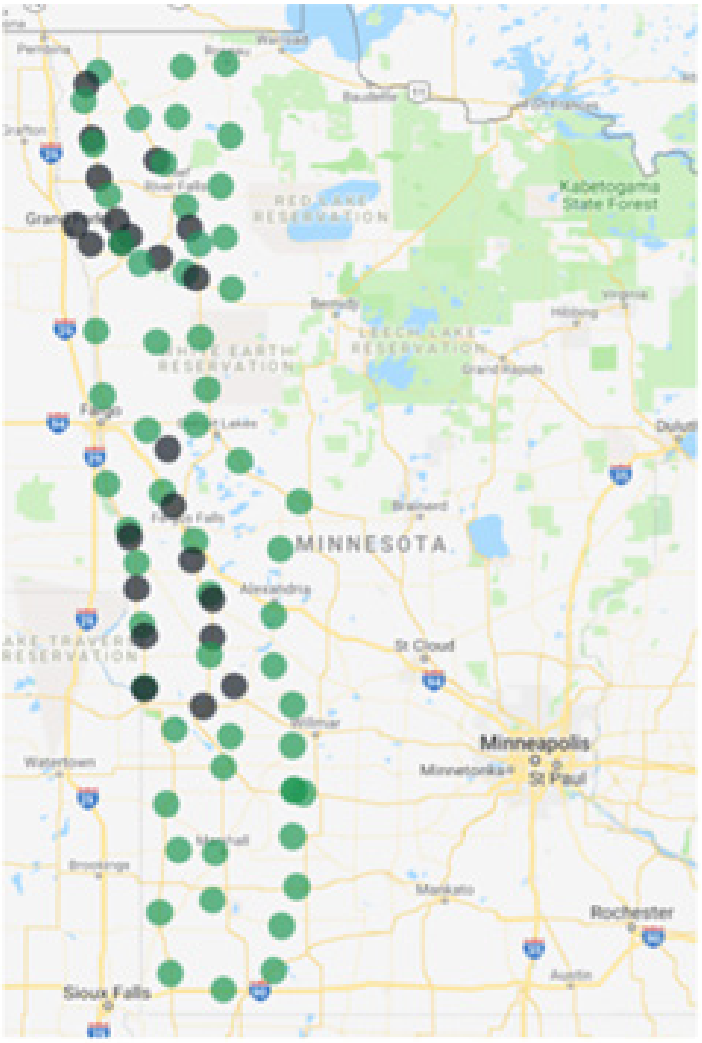

Fig. 1B: distribution of sampling locations for spring 2018 (black) and fall 2018

Publications *continued*

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Reynolds, S.K., Kolmer, J.A., Jin, Y., Rouse M., Dill-Macky, R., Smith, M.J., Hareland G.A., and Ohm, J.-B. (2018). Registration of 'Bolles' hard red spring wheat with high grain protein concentration and superior baking quality. *Journal of Plant Registrations,* 12:215-221.

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Reynolds, S.K., Kolmer, J.A., Jin, Y., Rouse M., Dill-Macky, R., Hareland G.A., and Ohm, J.-B. (2018). Registration of 'Linkert' spring wheat with good straw strength and adult plant resistance to the Ug99 family of stem rust races. *Journal of Plant Registrations*, 12:208-214.

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.

Anderson, J.A., Wiersma, J.J., Linkert, G.L., Reynolds, S.K., Kolmer, J.A., Jin, Y., Rouse M., Dill-Macky, R., Hareland G.A., and Ohm, J.-B. (2018). Registration of 'Norden' hard red spring wheat. *Journal of Plant Registrations*, 12:90-96.

Conference Proceedings

Ledman, K.E. ,Curland, R.D., Ishimaru, C.A., and Dill-Macky, R. (2018). Weedy grasses as a potential reservoir of the pathogen causing bacterial leaf streak of wheat. In: *Proceedings of the 11th International Congress of Plant Pathology*, Boston MA, USA, July 29-Aug 3, 2018. (Poster 1093)

Winter, M., Samuels, P.L., Otto-Hanson, L.K., Dill-Macky, R., and Kinkel, L.L. (2017). Biocontrol of Fusarium crown and root to in wheat by inhibitory Streptomyces. In: *Proceedings of the miCROPe 2017 International Symposium "Microbe-Assisted Crop Production - Opportunities, Challenges and Needs"*, Vienna, AUSTRIA, December 4-7, 2017, p. 137. (Poster: PT-MI-21)