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 continues our efforts to address diseases that impact the yield and quality of wheat in the Upper Great Plains, including Minnesota. The project focuses on three diseases; bacterial leaf streak (BLS); root and crown rots; and viral diseases that have been of increasing concern to wheat production in the state in recent years. The ultimate goal of the project is to deliver disease control measures for all three of these 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 is aimed as improving 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 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. 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. This survey work helped us prioritize research needs and has provided isolates needed for establishing screening for resistance to root and crown rots. Efforts in establishing a field screening nursery for root diseases have been challenging, but we have made progress in developing methods for screening plants against *Fusarium* spp. in the greenhouse in 2017. Greenhouse work has allowed us to identify sources of resistance and start screening breeding materials for reaction to the prevalent root rot pathogens in the region. 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. The data collected in previous projects gave us information on the strains prevalent in commercial crops around the state. In the 2017 growing season we sampled grass species adjacent to small grains fields along with the cultivated crop to examine the strains present in both for comparison. 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. 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 2017 we tested released varieties and advanced lines in a regional cooperative nursery (BLSCN). The 100 entries came from six wheat breeding programs (3 public [UMN, NDSU, SDSU] and 3 private [Bayer CropScience, Monsanto, 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 will be utilized by regional wheat breeding programs to the benefit of growers. Information on the response of released germplasm to BLS collected in 2017 will be combined with previous data sets and the overall evaluations will be disseminated to Minnesota growers through the MN variety trials bulletin. In 2017 we continued our work examining the role that wild grasses and other grass hosts play in the epidemiology of BLS in Minnesota. We made collections of symptomatic tissues and then isolated and identified the bacteria associated

with these host plants. From the sampled hosts, *Xanthomonas translucens* isolates were found on the following weed hosts: wild oat, smooth brome, quackgrass, foxtail barley, perennial ryegrass, and green foxtail and also from cultivated wild rice, intermediate wheatgrass, wheat, and barley. Molecular analysis of the isolates obtained indicated that the isolates from wheat, intermediate wheatgrass, wild rice, and the grassy weed hosts, except smooth brome, group with the pathotype strain *X. translucens* pv. *undulosa* and are thus considered to be pathogens of wheat. Isolates from smooth brome grouped with the pathotype strain *X. translucens* pv. *cerealis*. Isolates from barley grouped with the pathotype strain X. *translucens* pv. *translucens*. These groupings match the results of seedling assays, conducted in the greenhouse, and in a field experiment conducted in the summer of 2017, where the isolates of *X. translucens* were tested for their ability to cause disease on wheat and barley plants.

Root and Crown Diseases:
In 2017 we continued our work refining protocols to identity the fungi isolated from field surveys using molecular and/or DNA sequencing. In 2017 we tested a number of protocols to screen germplasm for resistance in the greenhouse and our efforts were met with success. The means that we now (finally) have some capacity to examine breeding materials for their response to crown rot. Given that our ability to screen materials in the field remains limited this was a positive finding. We have identified a wheat population that we believe is segregating for reaction to crown rot and plan to screen this population in 2018 to verify the putative reactions, identify progeny with improved resistance with the ultimate goal of identifying QTL for resistance.

Virus Diseases:
Results from our analysis of samples collected in Minnesota, along with those collected by our colleagues in North Dakota and South Dakota, 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 for over 10 years ago in the region. In our 2016 survey however, BYDV-PAV was found less frequently in other species, such as oat, and other strains such CYDV-RMV or CYDV-RPV seemed to be more common. More extensive sampling of other host species was undertaken in 2017 to determine if this finding is born out with a larger sample size. In the 2017 growing season, sixty six locations were sampled to address this question. RNA extraction prior to PCR detection for BYDV is under way in the lab and will continue over the winter months. This work will identify which, if any, of the grass weeds prevalent in Minnesota are infected with BYDV and if so, whether the strains in these weed species are similar to those present in small grains crops.

Application and 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 2017 we have both utilized protocols we have developed to establish screening nurseries for BLS, have improved screening protocols for BLS and the root diseases and have made significant progress in identifying sources of resistance to these diseases that will be of value in future breeding efforts.

Materials and Methods

Bacterial leaf streak: We have previously developed the basic protocols needed to work with BLS and 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 Drs Shaukat Ali (South Dakota State University) and 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, 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 laboratory and greenhouse methods for inoculating the roots and stem bases of wheat plants with *Fusarium* spp. These efforts continued in 2017 and have 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. Sixty-six locations were sampled in a Minnesota survey conducted during the 2017 growing season. Survey locations, ranged from the Canadian border to the Iowa state border and to the western border of the state adjacent to North Dakota. North of Highway 10 the majority of the ditches sampled were next to wheat or barley fields, with the occasional soybean field being sampled. South of Highway 10 sampling was generally conducted adjacent to corn and soybean fields, reflecting the primary crops in this region of the state, although sampling was occasionally conducted adjacent to oat fields. At each location samples (an average of 6-7 plants) were collected that were representative of the primary grass species growing in the ditches. As soon as possible after collection the sampled plants were identified and placed in the -80°C freezer for storage until RNA extractions, for virus identification, could be conducted. Samples from the northern part of the state were largely smooth bromegrass (*Bromus inermus*) and Kentucky bluegrass (*Poa pratensis*). In the southern half of the state samples of muhly grass (*Muhlenbergia richardsonis*) and quackgrass (*Agropyron repens*) were common though in certain locations little bluestem (*Schizachyrium scoparium)* were also sampled. RNA extractions, as is required for the PCR detection of BYDV strains, is under way in the lab and will continue over the winter months. This work will identify which, if any, of the grasses sampled are infected with BYDV and if so, whether these strains are similar to those present in small grains crops.

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. 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.

Research work on Bacterial Leaf Streak and Fusarium crown rot being presented at the Minnesota Wheat Research Update held at the Northwest Research and Outreach Center on July 18, 2017. Presenters (shown from left to right) were Kristi Ledman, MS graduate student working on host range studies of the BLS pathogen, Mark Winter, visiting scientist working on crown rot and inoculation protocols, and Rebecca Curland, researcher working on the BLS cooperative nursery.

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 benefitted from knowledge of 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 2018 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 continuing 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 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 though we have made steady progress in 2017 toward developing greenhouse protocols that are of value to breeding efforts. We plan to continue work in 2018 with a focus on additional phenotyping of germplasm.

Viruses: Efforts examining BYDV and CYDV in non-crop hosts will continue in 2018. We plan to complete our work determining 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 develop and validate a quantitative PCR method that we have a molecular-based assay for the detection of these viral strains. These efforts will be both informative to resistance breeding efforts and disease risk assessment.

Publications

Dill-Macky, R, Stanton, J.L. and Smith, M.J. (2017). Bacterial leaf streak: an emerging disease of wheat in the Upper Midwest of the United States. A*bstract in the Proceedings of Science Protecting Plant Health,* Brisbane, Australia, September 26-28, 2017.

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