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

1. PROJECT TITLE

Root Rot Diseases in the Upper Midwest: a coordinated approach to combatting this complex of diseases

2. PRINCIPAL INVESTITAGATOR (S)

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4. REPORT DATE

11/15/2013

5. REPORTING PERIOD

1/1/2013 to 12/31/2013

6. TERMINAL REPORT ____X_____ **PROGRESS REPORT** _____

7. AMOUNT OF GRANT $45,000

8. PUBLICATIONS

Gautam, P. and Ali, S. 2013. Root rot disease survey in South Dakota. Phytopathology 103(Suppl. 2): S2.48.

Gautam, P. and Ali, S. 2013. Evaluation of *Fusarium graminearum* isolates from wheat roots for their ability to cause crown rot in South Dakota. Phytopathology 103(Suppl. 2): S2.48.

9: EXECUTIVE SUMMARY

Research Question:

In 2012 we undertook the first research work on root rots of wheat in the Upper Midwest in over a decade. Given the lack of current research on root diseases we decided the most prudent approach was to begin with a survey to determine the prevalence of these root diseases to help establish their importance and the relative frequency of each of the likely pathogens. We also began the task of developing techniques to work effectively with these pathogens and toward establishing a management plan. The 2013 project represented a continuation of the original 2012 project and contains four specific objectives:

1) Survey root diseases incidence and severity in the three-state region
2) Identify and characterize the fungal pathogens associated with root diseases
3) Screen wheat lines for resistance to CRR and FCR
4) Develop effective methods for screening for resistance against root rot pathogens

Results:

1) We surveyed the spring wheat production area in each state in the 2013 cropping season for root diseases. In **Minnesota**, 28 fields were sampled, these augmented the 51 fields sampled in 2012 and were selected to fill in some gaps evident in the 2012 survey. Thus the 2013 survey allowed for a more complete sampling of the wheat production region in Minnesota for root rot pathogens. One hundred plants, 5 plants from each of 20 locations, were collected from each field. The GPS coordinates were recorded for all fields sampled and for almost every field the landowner was contacted to obtain production information for the field. In addition to the plant samples taken, we collected soil samples (3 cores) from each of three locations in each field surveyed. The collected plants were stored at 4°C until they could be processed. Fusarium crown rot (FCR) was observed in the majority of fields from which these collections were made, though the symptoms were not as readily evident as in 2012. Given the apparent prevalence of FCR in Minnesota, our research has initially focused on FCR and the isolation of *Fusarium* species from these samples. In **North Dakota** 206 wheat fields across 41 counties were sampled. We recorded the GPS coordinates of all fields sampled. In each field, 5 to 10 plants were collected and their root systems washed under tap water. The subcrown internodes (SCI) and crown tissues were examined visually for symptoms. A rating scale of 0 to 4 was used to record the disease severity on SCI, where 0, 1, 2, 3, 4 indicated 0, 20%, 50%, 75% and 100% of SCI with visual discoloration, respectively. Samples from most of the sampled fields had a disease severity of 4. In general, root disease incidence and severity were higher in 2013 than in 2012. The collected SCI and crown tissues with visual discoloration were stored at 4^{o}C until fungal isolations could be made. In **South Dakota** samples were collected from 24 wheat fields in 2013 in addition to the 50 fields sampled in 2012. Sampling locations covered both the spring and winter wheat growing regions in the eastern half of the state. One hundred tillers (5 tillers from each of 20 locations) were uprooted from each field. Most of the samples were collected arbitrarily, unless some plants exhibited root rot symptoms in which case these plants were preferentially selected. The collected tillers were washed under tap water to remove soil and stored at 4°C until used for fungal pathogen isolation and identification.

2) In **Minnesota** the root and crown tissues collected in 2013 have been dissected from the collected plants and are being used for the isolation and identification of the fungal pathogens associated with symptoms observed in the field. Our preliminary data from the 2013 survey

suggests that *Fusarium* spp. are the most common fungal pathogens present in the symptomatic tissues, as was the case in the 2012 survey. In **North Dakota** the tissues obtained during the field survey were used for the isolation of fungi, confirming the pathogens associated with the symptoms observed in the field. The 2012 samples have all been processed and we are still in the process of isolating fungi from the samples collected this year (2013). Among the 846 fungal isolates obtained from SCI and crown tissues, 302 (35.7%) were identified as *Fusarium* species, 121 (14.3%) were *Bipolaris sorokiniana* (common root rot) and 423 (50%) belonged to other fungal species. Of the 302 *Fusarium* isolates, *F. acuminatum*, *F. avenaceum*, *F. culmorum, F. graminearum*, *F. oxysporum*, *F. redolens*, and *F. solani* accounted for 16.9%, 11.9%, 5.3%, 7.9%, 24.8%, 12.6%, and 5.3%, respectively. We are testing pathogenicity of representative isolates from each of these species. In **South Dakota**, all root samples collected in 2012 and 2013 (74 total) were cut into small segments (~1-2 cm long) and washed thoroughly under running tap water, rinsed in distilled sterile water, and disinfected with a 5% bleach solution. Root segments were plated on ½ PDA for isolating *Fusarium* species and *B. sorokiniana* and on SM-GGT7 media for recovering *G. graminis* var. *tritici.* In 2012, *F. graminearum* and/or *F. acuminatum* were recovered from 15 root samples and *B. sorokiniana* was recovered from 10 root samples. Of the 35 root samples analyzed, *G. graminis var. tritici* was only isolated from one. Of the 24 root samples collected in 2013, 23, 8, and 8 samples produced *F. acuminatum*, *F. graminearum*, and *B. sorokiniana*, respectively, while 6 out of the 15 samples analyzed produced *G. graminis* var. *tritici.* In general, *F. acuminatum*, not previously considered to be an important root pathogen, was found to be the most prevalent *Fusarium* species associated with the root samples collected in both 2012 and 2013 in South Dakota. Twenty-five samples still need to be analyzed for *G. graminis* var. *tritici.*

3&4) In **Minnesota** we started work on this objective last Fall using the *Fusarium* isolates collected in 2012. Preliminary experiments were conducted examining to establish an inoculation protocol for seedlings in the greenhouse. The use of an inoculum layer, consisting of inoculum grown in a corn meal or coarse wheat flour substrate was tested. The inoculum was placed as a layer ½ inch above seeds of the test wheat cultivars that were planted in 'conetainers' containing a sterile soilless potting mix. The method was successful in generating infection and led to symptom development in the wheat cultivars examined. A number of planting depths (1-5 inches) were also tested to establish protocols for growing plants that produce the maximum length SCI possible. The work isolating and identifying the fungal pathogens from the 2013 survey started following harvest and we anticipate further work on developing screening techniques over the winter and spring of 2013/14. **North Dakota** tested two methods for screening wheat lines for reactions to the root rot pathogens at the seedling stage, the Petri plate method and the sand-cornmeal-inoculum method.

i. In the Petri plate method, spore suspensions (10^6 spores per ml) were prepared from the fungal cultures. Seeds of wheat lines to be tested were surface-sterilized, placed on two layers of filter paper placed in Petri plates (5 seeds per plate). The spore suspension (2 ml per plate) was added, the Petri-plate closed, packed in zip-lock bag and incubated in the dark for 2 days at 22^0C. After the initial incubation the plates were provided with alternating periods of light (14 hr) and dark (10 hr). The germination rate was assessed 3 to 6 days after plating. Shoot length was also measured on the 6^{th} day. In this test four wheat genotypes (Amidon, Briggs, ND652 and Len) were inoculated with four fungal species (*B. sorokiniana, F. culmorum, F. redolens, and F. oxysporum*). Three replicates were used in each experiment. The germination rate of Amidon, Briggs, ND652 and Len was 80%, 80%, 87%, and 60%, respectively, when inoculated with *B. sorokiniana*. The germination rate was 87%, 87%, 100% and 100% for the four genotypes, respectively, when inoculated with *F. culmorum*. Seedling length reduction rates, following inoculations with *B. sorokiniana,* were 8%, 15%, 0, and 23% for Amidon, Briggs, ND652 and Len, respectively. The relative reductions observed correspond to the known common root rot resistance levels of these wheat lines. For the inoculations with *F. culmorum,* seedling length reduction was only observed for ND652 (24%); the other three wheat lines had longer seedling lengths in the inoculated treatments. When the four wheat lines were treated with *F. redolens* and *F. oxysporum*, no significant

differences were observed between the control and the inoculated seedlings in all the parameters measured, suggesting that these two fungi are not root rot pathogens.

ii. For the sand-cornmeal-inoculum method, fungal cultures, grown on PDA for 5 days in alternating 12 hr of light and 12 hr of dark were transferred to a mix of sterile sand, cornmeal, and distilled water (9:1:2 ratio) and incubated for 12 days. During the incubation, the inoculum was swirled daily to ensure even growth. Plastic cups were used as containers for planting, with 15 g of sterile vermiculite added to the cup, followed by 15 g of the sand-cornmeal-inoculum, then the seeds and a final layer of sterile vermiculite. The cups were watered and kept in a humidifier chamber for 10 days with alternating 14 hr light and 10 hr dark. Seed germination, root growth, and visual browning of the root and stem bases were examined 10-12 days after planting. A 0-5 scale (0 resistant, 5 highly susceptible) was used for scoring the root and stem base browning. Using this method 10 wheat genotypes (Alsen, Amidon, Briggs, Coteau, Faller, Glenn, Len, ND652, Reeder and Steele-ND) were inoculated with *B. sorokiniana* and *F. redolens*, respectively. Three replicates were used in each experiment. The results indicate that Len, Steele-ND, Coteau and Faller had very low germination when infected by *B. sorokiniana* (Appendix 1). All the genotypes tested had similar height reductions compared to the control (without inoculum) except for Steele-ND, which did not germinate after inoculation (Appendix 1). No significant effect of *F. redolens* inoculation on germination, seedling height, and root discoloration of the wheat lines tested, confirmed the results of the Petri-plate test suggesting that this fungus is not a root rot pathogen.

In **South Dakota** 10 *F. graminearum* isolates were randomly selected from the 69 isolates recovered in 2012 and tested for their ability to cause Crown Rot. To achieve this objective, four hard red spring wheat cultivars, Alsen, Len, Oxen, and Wheaton were planted in plastic pots in randomized complete block design with three replications. Twenty day old seedlings were inoculated individually with spore suspension of all 10 isolates using the protocol described in Mitter et al. (2006). The isolates used in this study caused crown rot in all four cultivars tested. Len was significantly different in susceptibility then the other cultivars tested. Now that a protocol for screening wheat genotypes for FCR has been established we plan to screen more wheat genotypes in South Dakota, including those developed by SDSU wheat breeders, for their reaction to FCR and CRR.

Application/Use:

1) Survey of root disease incidence and severity in the three-state region: Given the time since the last comprehensive survey, and changes to cultivars and production systems, we believe this survey was necessary to determine the relative importance of root diseases so that we can prioritize future research efforts.

2) Identification and characterization the fungal pathogens associated with root diseases: The root and crown tissues obtained during the field survey are being used for isolation of pathogens to confirm the pathogens associated with symptoms observed in the field and to obtain a representative collection of pathogen isolates for future research.

3) Screen wheat lines for resistance to Common Root Rot (CRR) and Fusarium Crown Rot (FCR): We expect there to be some variation in the response of wheat varieties to CRR and FCR. By screening commercial cultivars and advanced breeding lines we hope firstly to identify varieties that may be highly susceptible to these root diseases. Identifying highly susceptible lines will be of value to growers who may then avoid planting these lines and to breeders who may avoid promoting highly susceptible germplasm within their breeding programs.

4) Develop effective methods for screening for resistance against root rot pathogens: Developing effective screens for resistance against root rot pathogens will allow us to work with these pathogenic fungi. We need to be able to conduct inoculated experiments to evaluate any control strategy, including the use of host resistance.

Materials and Methods:

1) Survey root diseases incidence and severity in the three-state region: Each participating state took responsibility for conducting a comprehensive statewide survey of the spring and winter wheat production areas in the 2012 and 2013 cropping season. At least 50 fields were systematically sampled in each state to try and ensure geographic coverage of the entire wheat production region. Root and crown tissues were observed for symptoms and the fields sampled to determine the incidence and severity of root diseases. Samples collected at each site were bulked, the root systems of sampled plants washed to remove soil prior to storage. Plants and tissue samples are being stored at 4^{o}C until we are able to process the sampled materials to recover fungal pathogens.

2) Identify and characterize the fungal pathogens associated with root diseases: The root and crown tissues obtained during the field survey are being used for isolation of pathogens to confirm those pathogens associated with symptoms observed in the field. The isolates obtained have been added to the collections of pathogens in each state. Each state has undertaken isolation and identification of fungal pathogens associated with root diseases. The root, SCI and crown tissues from the collected plants will be dissected and used for isolation of pathogens to confirm those pathogens associated with symptoms observed in the field. Selective media is being utilized to recover pathogens where multiple pathogens may be present. For example PDA media amended with streptomycin can be used to isolate *Bipolaris sorokiniana*, Komada's media or specific screening media (SSM) has been used to isolate *Fusarium spp. and* modified SM-GGT3 media has been used to isolate *Gaeumannomyces graminis* var. *tritici*. Isolated fungi are initially being identified based on morphological characteristics. We plan to confirm the identities of these isolates using genetic tests following DNA extraction. Each state has tested the pathogenicity to wheat of the recovered fungal pathogens. These inoculation tests are generally being conducted on wheat plants in the greenhouse.

3) Screen wheat lines for resistance to CRR and FCR: We know that there is some variation in the response of wheat varieties to CRR and FCR and so we will initiate the screening of commercial cultivars and advanced breeding lines. Each state will screen approximately 20 hard red spring wheat (HRSW) genotypes for reaction to *B. sorokiniana* in the greenhouse. Each state will select lines representative of the germplasm in their state, including a number of lines with a known response to CRR and with sufficient common entries such that the data from each state can later be compared. In addition SDSU will also examine hard red winter wheat cultivars for their response to *B. sorokiniana.* Each state will each screen approximately 20 wheat genotypes for reaction to *F. graminearum* (FCR) in the greenhouse. States will select lines representative of the germplasm in their state, including a number of check lines with a known response to FCR. With all tests we have agreed to include sufficient common entries such that the data from each state can later be compared.

4) Develop effective methods for screening for resistance against root rot pathogens: We plan to examine, as necessary, inoculation methods for root diseases including CRR and FCR. If significant levels of take-all are identified we will also work to establish effective inoculation methods suitable for greenhouse screening for take-all.

Economic Benefit to a Typical 500 Acre Wheat Enterprise:

The preliminary data gathered from our surveys in 2012 and 2013 indicate that root rot diseases are prevalent in commercial wheat fields in Minnesota, North Dakota and South Dakota. It appears that the two pathogens we anticipated finding, *Fusarium* and *Bipolaris,* are indeed abundant and likely contributing significantly to yield losses. In Minnesota we estimated that in 2012 over 20% of tillers had died prematurely - these were visible as 'white heads' in the field - as a result of root disease in a the most severely impacted fields. It is clear that root diseases have also been widespread in 2013. While this information does not provide any immediate

benefit to the grower an awareness of the problem is a critical first step if control measures to root rots are ultimately to be implemented.

10: RELATED RESEARCH

This coordinated project represents the main research effort on the root diseases of wheat in the Upper Midwest and fits alongside a study being conducted by Dr. Madeleine Smith on efficacy of seed treatments for root and crown rots. We hope that together these studies provide and integrated approach to understanding root rot diseases and that they are building a foundation for developing effective control practices.

11: RECOMMENDED FUTURE RESEARCH

This collaborative survey has demonstrated that root rot pathogens are readily found in association with wheat plants in the Upper Midwest and likely have negative impacts on wheat yields. We recognized that the root rots, and the soil-borne pathogens that incite them, are inherently more challenging to work with than foliar diseases and thus this collaborative effort has proven effective in taking the initial steps to understanding the prevalence of these pathogens. Sampling fields and processing the plant samples we collected has taken us longer than we initially anticipated, however despite making slow progress we believe we are gaining momentum and have already developed some insights of the problem root rot disease pose to wheat production in the Upper Midwest. We anticipate completing the isolation of fungal pathogens from the remaining samples collected in the surveys and plan to complete identifying the fungal isolates using morphological and/or DNA sequencing. We have made significant progress in testing methods suitable for inoculating plants with *Bipolaris sorokiniana* and *Fusarium* spp. in the greenhouse that has facilitated our ability to complete the remaining objectives of the current study. We plan to continue with a collaborative research approach focusing on understanding the response of the wheat germplasm to these pathogens. We will focus both on identifying highly susceptible varieties that may be driving disease and to identify lines with higher levels of resistance that may ultimately be utilized in the development of varieties with improved tolerance to these root pathogens.

Specific objectives for future research include:

1) Finish fungal isolation from the sub crown internodes and crown samples collected in 2013.
2) Characterization the isolated fungi based on morphological characteristics, DNA sequence, and pathogenicity tests.
3) Optimize methods for screening wheat for reaction to root rot pathogens, both in the greenhouse and field.
4) Screen commercial cultivars and advanced breeding lines for resistance to CRR and FCR.
5) Conduct limited field surveys, principally to sample ‘hot spots’ for root rots identified in the 2012 and 2013 surveys, and to sample underrepresented areas including winter wheat production fields.
6) Examine the efficacy of seed treatments for the control of root and crown rots. Work on this objective was started in a separate project, lead by Madeleine Smith, however we envisage this work proceeding under the auspices of the Upper Midwest Wheat Pathology Collaboration (UMWPC) in 2014.

12: APPENDIX

Appendix 1: Variety response (germination, height reduction and disease development) following inoculation with an isolate of *Bipolaris sorokiniana* in comparison to non-inoculated controls.

Variety	B. sorokiniana Isolate	Germination (%)	Seedling Height Reduction (%)	Disease Reading (1-5 scale)
Alsen	120-C-3 (Bs)	20.0	67	3
Amidon	120-C-3 (Bs)	53.3	67	3
Briggs	120-C-3 (Bs)	46.7	67	5
Coteau	120-C-3 (Bs)	0.6	58	4
Faller	120-C-3 (Bs)	0.6	61	5
Glenn	120-C-3 (Bs)	33.3	66	4
Len	120-C-3 (Bs)	0.6	64	5
ND652	120-C-3 (Bs)	53.3	70	3
Reeder	120-C-3 (Bs)	33.3	53	3
Steele-ND	120-C-3 (Bs)	0.0	100	No data

Data from North Dakota State University, 2013.