A Coordinated Research Plan to Address Bacterial Leaf Streak

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Research Question

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.

Results

1) In greenhouse experiments at NDSU, plants infiltrated with needle-less syringe gave the highest infection in the greenhouse. In addition, inoculation of flag leaves of spring wheat line ND 495 with inoculum dose (10⁷ cfu/ml) exhibited high BLS symptoms between 7 and 10 days after infiltration.

SDSU compared injection methods with high and low-pressure spray techniques in the greenhouse. It was determined that the low-pressure spray techniques provided adequate infection. Disease development was however dependent on subsequent incubation under high-moisture environments.

All inoculation techniques examined in a St. Paul field trial were effective in establishing BLS in the field plots. The spray inoculation applied late in the growing season (heading) generated more disease than when the inoculum was applied early (tillering). The needle inoculation, which was applied before the earlier of the two spray inoculations, generated a level of disease that fell between those from the spray inoculation treatments. Inoculum concentration influenced disease development but was less important than the timing of the inoculation. The response of the wheat cultivars examined was consistent across all treatments and the two isolates used. This finding suggests that there is not a cultivar by isolate interaction in this host-pathogen interaction.

The correlations among the three assessment techniques used to assess BLS in the St. Paul trial were high (correlation coefficients ranged from 0.72 to 0.81). This finding indicates that differences among the assessment techniques used in different trials, different locations, by different researchers will be comparable even if there are differences in the assessment scales used. This is encouraging as it means that research results from any state / program / researcher should be transferable to other locations and that BLS assessment techniques may be selected and employed as appropriate to a specific trail’s objective. For example, a whole plot score might be appropriate for scoring breeders plots where large numbers of plots are to be assessed while scoring leaves on a number of tillers within a plot might be better suited to understanding disease development and assessing yield losses from varying levels of BLS infection.

2) Despite strong levels of background disease in SDSU’s non-inoculated plots, a significant difference was noted for several of the varieties inoculated with *Xanthomonas* when compared to the non-inoculated comparison plots. Yields were reduced in inoculated plots by around 10-30% compared to the non-inoculated plots. Keeping in mind that there was a significant background level of disease in all plots, it is possible BLS may be capable of reducing yield potential even more than was observed in our study under high-pressure environments. Test weight was not highly affected in our preliminary experiment, however test weights for the entire study were very low. We believe that the disease would likely impact grain fill period and consequently, grain weight.

3) A variety of bacterial isolates with pathogenicity to wheat were collected and characterized by SDSU including a number of *Xanthomonas* spp. and also *Pseudomonas* spp. All isolates were subjected to pathogenicity testing in the greenhouse and several promising strains were selected for use subsequent inoculation studies.

UMN developed a collection of 133 disease-associated isolates from 12 locations in Minnesota, North Dakota and Montana. Thirty-three *X. translucens* isolates as well as a number of *Curtobacterium* spp. and *Pseudomonas* spp. were confirmed via media testing and DNA sequencing. Sixty isolates from the 2009 field season were also characterized via the same methods as the 2010 collection and 13 X.
translucens isolates were confirmed. All isolates were stored in glycerol stocks at -80˚ C and will be tested for pathogenicity during winter 2010 and spring 2011.

NDSU conducted virulence tests with a total of 266 strains of X. t. pv. undulosa. Results showed that the majority of strains isolated from wheat (i.e., wheat strains) induced symptoms on both wheat and barley cultivars. The strains isolated from barley (i.e., barley strains) also infected wheat but barley strains were less virulent than wheat strains. Ten randomly selected strains were infiltrated on wheat (ND 495), barley (Robust), oat (Ajay), rye (AC Remington) and triticale (PI 634537) flag leaves. Only a few strains were avirulent on oat, rye and triticale, but all strains were virulent on wheat and barley.

4) SDSU identified a resistant line – SD4205 – as well as a couple of intermediate lines and several highly susceptible ones. Data collected over four location-years has shown the repetitability of the inoculation techniques and confirmed the preliminary results for each line in the tests.

NDSU found that about 8.3% of accessions tested from a set of 605 winter wheat accessions were resistant. Accessions with an improvement status of ‘cultivar’ were significantly more likely to be resistant than were accessions classified as either landraces or breeding lines. Forty-two of the accessions resistant to strain BLSW16 were randomly selected and further evaluated utilizing additional two strains BLS Cr25 and BLS Lb74 of X. t. pv. undulosa from Car- rington and Lisbon, respectively. Nonparametric data analysis revealed 35 accessions were resistant to all three strains tested while six accessions showed differential responses. Among a second screening of 567 spring wheat accessions, nearly 31% of the accessions were resistant to BLS. These accessions were further genotyped with 832 Diversity Array Technology (DArT) markers and utilized an association mapping approach to identify resistance loci for X. t. pv. undulosa. Eight putative candidate QTL regions were identified and may be useful in wheat breeding programs.

Application/Use

1) 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, which relies on screening to identify sources of resistance, and introgress resistance into adapted germplasm.

2) Although yield/loss data for BLS are currently unclear, preliminary studies have indicated that BLS is of economic importance. Further experiments are necessary to understand how infections either early on leaves or later on leaves and heads impact the yield components of wheat. These studies are essential to justifying the cost of developing a disease control program.

3) 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. Determining if there are ‘races’ of the pathogen is 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.

4) Although a cooperative screening nursery for commercial lines has not yet been developed, the data produced by our preliminary screenings have identified lines with resistance and with high susceptibility. These data can be applied to breeding efforts and lead to the development of commercially available resistant lines. Plans are in progress to launch our cooperative screening nursery over the 2011 field season. Initially this nursery would screen commercially available wheat cultivars (public and private releases) to provide information on relative variety performance to growers. Nurseries to screen breeding lines will be developed as necessary.

Materials and Methods

1) A variety of inoculation methods were tested in the greenhouse (NDSU, SDSU) and in the field (UMN, SDSU). Techniques that were tested include leaf infiltration using a needle-less syringe, needle injection, pin-pricking, rubbing, leaf clipping and foliar spray. All inoculation methods were also studied in respect to bacterial isolate, inoculum concentration and plant growth stage.

The field trial conducted in St. Paul examined several variables that likely influence BLS development including; bacterial isolate (two isolates SY & Xtt), inoculum concentration (10^6 & 10^7 CFU/ml), inoculation technique (needle and spray), timing of inoculation (tillering and heading). A mist-irrigation
system, similar to that used in FHB screening nurseries, was utilized to facilitate infection and disease development. Disease development in control plots suggested that background infection was quite low.

Three rating systems were examined: a whole row score (1-9 scale, one value per plot), a whole plant score (0-9 scale, 10 plants/plot) and a flag leaf score (percent scale, 10 plants/plot). Plots that were spray inoculated were assessed 10-11 days after inoculation while the needle inoculated plots were assessed 21 days after inoculation.

At SDSU, a double-digit rating system was employed to assess both the severity of infection on leaves and also the height or vertical movement within the canopy. Field trials were monitored and assessed four times at one-week intervals from boot stage through late milk/early dough stage. A severity value was obtained for each assessment data point and from that data, area under the disease progress curve (AUDPC) could be calculated and an assessment of rate of progress was made for each line in the tests.

2) A set of plots was established by SDSU to estimate production losses due to BLS. Five lines were utilized in paired studies – inoculated compared to non-inoculated.

3) The bacterial isolates with pathogenicity to wheat that were characterized by SDSU in 2010 included a number of *Xanthomonas* spp. and also *Pseudomonas* spp. These isolates were collected from field infections of wheat in South Dakota in 2008 and 2009. Isolates were identified by cultural and biochemical tests as well as with the Biolog™ Identification System – a nutrient utilization profile with sub-species level capabilities. All isolates were subjected to pathogenicity testing in the greenhouse and several promising strains were selected for use in subsequent inoculation studies.

At NDSU, to determine virulence in *X. t. pv. undulosa*, a total of 266 strains from Carrington (n = 45), Casselton (n = 32), Langdon (n = 76), Lisbon (n = 57), and Prosper (n = 56), were infiltrated (10⁷ cfu/ml) on wheat (ND 495) and barley (cv. Robust) flag leaf in the greenhouse. To examine genetic diversity in *X. t. pv. undulosa*, the five populations (total = 266 strains) collected from Carrington, Casselton, Langdon, Lisbon, and Prosper in North Dakota were analyzed using two repetitive sequence-based primers (ERIC and BOX) and an insertion element, IS111, from *X. oryzae pv. oryzae* (IS-PCR) with J3 primer. High gene flow and low population differentiation was observed between and among five populations of *X. t. pv. undulosa*.

UMN strains were initially isolated on Wilbrink’s Agar or Nutrient Agar and subsequently screened on XTS (a semi-selective medium for *X. translucens*). All putative *X. translucens* isolates were confirmed via PCR *X. translucens*-specific primers and sequencing with 16S primers.

4) Two separate experiments (for winter wheat and spring wheat) were conducted to evaluate resistance to BLS in the core subset of the USDA National Small Grain Collection (NSGC) in NDSU’s greenhouse. In the first experiment, a set of 605 winter wheat accessions of diverse origin were initially infiltrated (10⁷ cfu/ml) with a virulent strain BLSW16 of *X. translucens pv. undulosa* from Casselton, North Dakota (ND) on the flag leaf of each plant. Disease reactions were assessed between 7 and 10 days after infiltration using a 0 to 6 rating scale, where <2.0 was considered as resistant and >2.0 was regarded as susceptible. In the second experiment, 567 spring wheat accessions collected from diverse geographic areas were evaluated against a strain BLSW16 of *X. t. pv. undulosa* in the greenhouse. All inoculation and disease scoring methods were the same as described above.

**Economic Benefit to a Typical 500 Acre Wheat Enterprise**

At this time there is no direct economic benefit of the research, although our preliminary understanding of this disease has enabled us to provide growers with information on those cultivars that are most resistant and susceptible to BLS. We anticipate that this information, in conjunction with information on the potential yield losses caused by BLS will enable us to better quantify the impact of this research effort.

**Related Research**

As a three-state coordinated effort this work dovetails into other research at each of the cooperating institutions.
At the meeting held in Moorhead MN this past September we established a cooperative effort, with the wheat breeding programs (public and private) in each of the states, to assess BLS in fungicide-treated yield trials where natural infections of the disease occur. The purpose of this is to establish a database that will provide information on the response of commercially grown wheat cultivars to BLS and thus be more readily able to transfer that information to growers.

**Recommended Future Research**

Continued work has already been anticipated in the FY11 pre-proposal which has already been submitted to the MNWR&PC. At this time our objectives have largely stayed the same, with the FY11 project confirming many of the preliminary findings that were outcomes from the FY10 project, although the new project expands our ability to test commercial wheat cultivars for their response to BLS.

**Publications**

