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Reducing soybean cyst nematode, *Heterodera glycines* populations by planting cover crops in infested soils

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Soybean cyst nematode (SCN; *Heterodera glycines*) is one of the major soybean pests. Cover crops were evaluated in the greenhouse and microplot by using naturally infested soils from two fields in Richland and Cass counties, ND. SCN did not reproduce in annual ryegrass (*Lolium multiflorum* L.), camelina (*Camelina sativa* Crantz.), Ethiopian cabbage (*Brassica carinata* L.), faba bean (*Vicia faba* Roth.), foxtail millet (*Setaria viridis* L.), radish (*Raphanus sativus* L.), rape (*B. napus* L.), red clover (*Trifolium pratense* L.), sweetclover (*Melilotus officinalis* L.), triticale (x *Triticosecale* Whitt.), and winter rye (*Secale cereale* L.). Cowpea (*Vigna* spp.), crimson clover (*T. incarnatum* L.), and turnip (*B. rapa* L.) had low SCN reproduction (white females: 1 to 13). All pea (*P. sativum* L.) types and hairy vetch (*V. villosa* Roth.) showed some reproduction (14 to 173) compared to susceptible checks (827 to 1,251). Furthermore, 10 cover crops were planted in microplot pots each containing 5 kg of soil from each field under natural conditions. After 75 days of growth, soil samples were collected and analyzed for reproduction factor (RF: final/initial population) of SCN. All the tested cover crops except Austrian winter pea significantly ($P < 0.0001$) reduced the SCN populations (RF: 0.27 to 0.74). Annual ryegrass and radish had the greatest population reduction (RF: 0.27 to 0.33) and will be used for understanding mechanisms of population reduction in further field trials.

Fungicide seed treatments for evaluating the corn seedling disease complex following a winter rye cover crop

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A complex of pathogens including *Pythium* and *Fusarium* species, and *Rhizoctonia solani* cause seedling disease of corn. The goal of this research was to improve our understanding of this complex. We used seed treatments to manage genera in the disease complex and compared disease development, pathogen prevalence and seedling growth. Since risk of seedling disease is greater after a winter rye cover crop, a rye treatment was included. The following fungicide treatments, at recommended rates, were compared with and without rye in the growth chamber (i) ALL, metalaxyl, pyraclostrobin fludioxonil, ipconazole, and sedaxane, to reduce infection by *Pythium* and *Fusarium* species, and *R. solani* (ii) P, metalaxyl alone, to reduce *Pythium* (iii) FR, pyraclostrobin, fludioxonil, ipconazole and sedaxane, to reduce *Fusarium* and *R. solani* (iv) FP, metalaxyl and pyraclostrobin, to reduce *Fusarium* and *Pythium*, and (iv) NF, non-treated seed control. Seed treatments improved seed germination and seedling growth in both runs ($P < 0.05$). In run 1 only, recovery of *Pythium* spp. was reduced in P, FP, and ALL with rye ($P = 0.025$) and without rye ($P = 0.020$). No effect of seed treatments on recovery of *Fusarium* spp. was detected in both runs. Overall, better seedling growth and reduced *Pythium* incidence was observed in ALL, P, and FP compared to FR and NF. Data from this study support earlier studies that *Pythium* species play a major role in the corn seedling disease complex.

Bacillus strains as plant growth-promoting agents for the Great Plains agricultural region

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The application of plant growth-promoting rhizobacteria (PGPR) has potential for disease biocontrol and to supplement inorganic fertilizers. However, the efficacy of a PGPR strain might depend on the crop it is applied to or the environmental conditions in which the crop is grown. The objective of this study was to identify endospore-forming PGPR strains that could promote the growth of multiple crops typical of the Great Plains region. Twelve strains of endospore-forming bacteria were isolated from the rhizosphere of wheat (*Triticum aestivum*) grown in Nebraska and were identified as species of *Bacillus*, *Paenibacillus* and *Lysinibacillus* via 16S rDNA sequencing. Greenhouse pot experiments using corn, wheat and soybean were conducted to assess their growth-promotion potential. *Bacillus megaterium* strain R181 (GenBank Accession number KY807994) increased the biomass of all three test plant species over the no-bacteria control. The other strains increased growth in only one or two of the plant species or had no effect on plant growth. All strains were screened for physiological traits associated with plant growth promotion including in vitro antibiosis, protease activity, biosurfactant production and phosphate solubilization. None of the strains inhibited the growth of fungi or Gram-negative bacteria, and only strain R181 was antagonistic to Gram-positive bacteria. All strains were positive for biosurfactant activity, most were positive for protease activity, but only three solubilized phosphate. None of the physiological traits were predictive of growth-promotion efficacy exhibited in the pot experiments. This study identified one endospore-forming strain with the potential to stimulate growth in multiple crops.

Impact of foliar fungicide timing on northern corn leaf blight severity and yield in corn

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Foliar fungicides are commonly used to protect corn plants from *Exserohilum turcicum*, the causal pathogen of northern corn leaf blight (NCLB). However, the disease is often not visible or severe until after tasseling, and questions remain about the impact of post-tassel fungicide applications on disease severity and yield. Research was conducted in 2014 and 2015 in Tippecanoe County, Indiana, to determine optimal fungicide timing and examine the risk associated with late season disease development. Experiments were inoculated with *E. turcicum* at the eight collar growth stage (V8) and again at the eleven collar growth stage (V11). A conidial suspension was applied over the top of corn plants at a rate of 1.2×10^6 conidia per plot. Fungicide was applied to the experiment at one of five timings: Tasselling/silking (VT/R1), blister (R2), milk (R3), dough (R4), and dough (R4) + 2 weeks. Disease severity assessments were taken at early dent (R5) by estimating the percentage of the ear leaf with symptoms of NCLB. In 2014, disease severity and yield were significantly affected by fungicide timing ($P = 0.001$, $P = 0.0217$, respectively), with significantly less disease observed in treatments where fungicide was applied at R1 compared to the non-fungicide treated control. In 2015, disease severity was affected by fungicide timing ($P = 0.0346$), with fungicide applications occurring at VT/R1 and R2 reducing disease compared to the non-fungicide treated control. Fungicide applications occurring after R2 in either year did not reduce final disease severity. These results indicate that under high disease pressure, fungicide applications should occur at VT/R1 and not past R2, to reduce disease severity and improve yield.

Characteristics of resistance to seed and root rots caused by *Pythium ultimum* and *Pythium irregulare* in early maturity soybean lines

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Soybean seed and seedling rots caused by *Pythium* species are a problem throughout Minnesota. Infection by *Pythium* spp. interferes with stand establishment, which can necessitate replanting and lead to reduced yields. Of more than 30 *Pythium* and *Phytophthora* species found in Minnesota *P. irregulare*, and *P. ultimum*, are most prevalent and most damaging as causes of seed and seedling rot. Cultivar resistance would offer an inexpensive, effective approach to disease management. The objectives of this study are: 1) to determine if resistance to seed decay is correlated with root rot resistance, and 2) to identify soybean genotypes resistant to *P. irregulare* (PI) and *P. ultimum* (PU) among soybean lines that are parents of Minnesota adapted soybean varieties. A population of 280 MG 00 - I soybean lines that are ancestral to the Minnesota soybean breeding program were evaluated for response to inoculation with PI and PU. A subset of 12 lines was evaluated for its response to inoculation with PI and PU observed in-vitro for seed rot and in growth media for seedling rot. Seed rot was poorly correlated between PU and PI inoculated lines ($n = 48$, $r^2 = 0.19$, $P = 0.63$) and sensitive to seed quality. Visual necrosis scores, and root mass or root length were negatively correlated ($n = 48$, $r^2 = -0.60$ & -0.75 , $P = 0.0001$ & 0.0009). Among the 280 lines assessed for response to PU there were significant differences in visual necrosis and root length indicating that differences in susceptibility to seedling rot caused by PU were present among those lines.

Impact of winter rye cover crop on soybean root disease and productivity

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Cover crops are known to improve soil and water quality but few studies have addressed their impact on soybean disease and productivity. The effect of winter rye cover crop on soybean health was studied at four experimental sites: ADW (IA) and BREC (MO),

in 2012 and 2013; and Boyd (IA) and ISUAG-USB (MO), in 2015 and 2016. The treatments were rye and no-rye, at ADW, BREC and Boyd; and early-kill rye, late-kill rye, and no-rye, at ISUAG-USB. Dry root weight, root rot severity and yield were assessed at all locations. Plant population and canopy reflectance were assessed at Boyd and ISUAG-USB only. Rye did not affect soybean dry root weight and root rot severity at any location or year. At Boyd, no-rye resulted in greater yield and plant population than rye in 2016 only. At ISUAG-USB, in 2015, no-rye showed greater yield than late-kill rye, and plant population was higher in no-rye and early-kill rye than late-kill rye; in 2016, no-rye resulted in lower yield and plant population compared to early and late-kill rye. At ADW and BREC, yield was not affected by rye treatments. At the end of the season, soybean canopy reflectance was greater in no-rye than in rye treatment at Boyd, and greater in no-rye than late-kill rye at ISUAG-USB. In these field studies, the use of rye did not affect soybean root disease, and soybean yield was inconsistent. Low disease intensity, high rye biomass in 2016, and other specific factors of each location may have influenced the results.

Evaluation of weed species as alternative hosts of soybean cyst nematode in South Dakota

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Soybean cyst nematode (SCN; *Heterodera glycines*) causes an estimated \$1 billion in revenue losses annually in USA and consistently ranks as the most threatening pathogen for soybean. Winter annual weeds can further exacerbate the SCN problem by harboring SCN in the absence of soybean in the field. These weeds have become widespread in recent years due to an increase in conservational tillage practices and the reduction in the use of residual herbicides. Identification and evaluation of alternative weed hosts of SCN is important to provide effective management strategies against SCN. To determine alternative annual winter weed hosts in South Dakota, 133 whole weed samples were collected during fall 2016 comprising 37-weed species from 11 fields that were previously infested with SCN. The weed species were soaked in water for 48 hours to separate the adhered soil from the roots. The roots were dissected into smaller pieces of 2 cm length, macerated in a blender at 12,000 rpm and the resulting suspension was passed through the series of 250, 150 and 45 μ m sieves. Using a dissection microscope, the filtrate was then examined within a counting dish. Based on the morphological features, SCN juveniles were found on the field pennycress (*Thlaspi arvense*), buffalobur (*Solanum rostratum*), common mallow (*Malva neglecta*), poppy mallow (*Callirhoe involucrate*), venice mallow (*Hibiscus trionum*), leafy spurge (*Euphorbia esula*), clover (*Trifolium repens*) and horseweed (*Erigeron Canadensis*) weed species. Preliminary findings from this study indicate these weeds are alternative hosts for SCN in South Dakota.

Inoculation timing by hybrid field trials to assess the impact of Goss' wilt and leaf blight on northern corn yields

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Goss' wilt and leaf blight of corn, (*Clavibacter michiganensis* subsp. *nebraskensis* (Cmn)), was first documented in North Dakota (ND) in 2011 and now is found in 19 counties. Given the prevalence of this disease across the state, potential yield loss attributed to Goss' wilt needs further investigation in northern corn hybrids. Field trials were established in 2015 and 2016, across four locations to assess yield loss attributed to Cmn inoculation timing and hybrid susceptibility. Trials were designed in a randomized complete block with a split-plot arrangement and four replications. Hybrids were planted as whole plots, with inoculation timings serving as sub-plots. Three corn hybrids, with sub-88 day relative maturities, and Goss' disease ratings of susceptible, moderately susceptible and tolerant were sown. Inoculation timings included a non-inoculated check, V6-V8 (early-season), R1 (late season), and a sequential inoculation of V6-V8 and R1 (combination). Beginning 14 days after inoculation, percentage disease severity was assessed for every plant in each plot every two weeks until corn reached maturity. At harvest, test weight and yield were recorded. Year-end disease severity values ranged from 8–37% on susceptible hybrids across locations. At one location, yield loss values in excess of 30% were associated with an early season inoculation. Results from this research will help create awareness and influence management decisions for ND corn growers.

Effect of a bionematicide seed treatment containing *Pasteuria nishizawae* on *Heterodera glycines* reproduction and soybean yield in Iowa from 2014–2016

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The bionematicide seed treatment Clariva Complete Beans, CCB (Syngenta), containing the bacterium *Pasteuria nishizawae*, has recently become available to supplement genetic host resistance for management of the soybean cyst nematode (*Heterodera glycines*; SCN). The effects of CCB on SCN reproduction and soybean yield were studied in experiments using 4-row-wide by 17-ft-long plots from 2014 through 2016 at nine locations each year across Iowa. Two treatments were compared: CruiserMaxx Advanced + Vibrance (CM+V) and CCB (CM+V plus 10^7 *P. nishizawae* spores per seed). A moderately resistant variety with the PI 88788 source of resistance was used in 2014, and resistant varieties with PI 88788 resistance were used in 2015 and 2016. SCN population densities were determined from soil samples collected in each plot at planting (P_i) and harvest (P_f) and were used to calculate a mean SCN reproductive factor (P_f/P_i) for each treatment in each experiment. Yield data also were collected. Significant reductions in SCN reproductive factor were observed at two locations with the CCB treatment relative to the CM+V treatment. Significant yield increases were not observed at any location in any year. A significant yield reduction of 1.4 bu/acre (94.2 kg/ha) was observed in the CCB treatment at two locations in 2015 and 0.7 bu/acre (47.1 kg/ha) when data from all locations were pooled in 2015. In these experiments, CCB was variable in its effects on SCN reproduction and soybean yields.

Testing the efficacy of bicarbonates as fungicides against *Cercospora beticola*

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Cercospora leaf spot (CLS), caused by the fungal pathogen *Cercospora beticola*, is an economically important pathogen of sugar beets in many production areas throughout the world. The application of fungicides has been one of the most effective management tools for CLS, but their effectiveness has diminished due to fungicide resistance. To manage CLS in the future, the development of new fungicides will be critical. One group of chemicals that may be effective against CLS is the bicarbonates, particularly potassium, sodium, and ammonium bicarbonate. Previous studies have shown these chemicals can reduce disease severity with *Sphaerotheca fuliginea*, *Botrytis cinerea*, *Venturia inaequalis*, and other fungal species. To test their efficacy against *C. beticola*, lima bean agar plates amended with either 70% ethanol as a control or one of the bicarbonates plus 70% ethanol for a final concentration of 1% (ethanol v/v, bicarbonate w/v) were prepared. A plug of *C. beticola* was transferred to each plate, and the diameter of the fungal colony was periodically measured with a caliper and recorded starting five days after the transfer. After seven days, the plates treated with potassium bicarbonate showed nearly a 50% reduction in growth compared with the ethanol plates. As other fungi vary in sensitivity to the form of bicarbonate, testing of the other forms is ongoing. Based on these findings, it is possible that bicarbonates could be useful to manage *C. beticola* in the field.

Effect of fungicide seed treatments against tan spot of wheat

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Tan spot, caused by *Pyrenophora tritici-repentis*, is an important disease of wheat causing 5% grain yield loss in South Dakota but individual field grain yield loss can be higher. *P. tritici-repentis* infections can start in the fall in winter wheat especially during extended periods of extended warm weather. Some producers are using fungicide seed treatments to manage foliar fungal diseases that develop in winter wheat in the fall. However, there is a paucity of information with regard to fungicide seed treatments in winter wheat to suppress fungal diseases in the fall. To determine the efficacy of fungicide seed treatments in managing tan spot, the tan spot-susceptible wheat cultivar 'Select' was subjected to three seed treatments: ipconazole + metalaxyl, triticonazole + metalaxyl, and a non-treated check. Treated and non-treated seeds were planted into "cone-tainers" under greenhouse conditions. One week after planting, all plants were inoculated with *P. tritici-repentis* at a concentration of 3,000 conidia ml⁻¹ until run-off and placed in a misting chamber for 24 hours before returning to the greenhouse. Plants were assessed for tan spot severity at 4, 7, and 14 days after inoculation. Preliminary results indicate no significant difference between seed that was fungicide treated and the non-treated check for tan spot severity. Further experiments to establish the efficacy of fungicide seed treatments against powdery mildew (*Blumeria graminis* f. sp. *tritici*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) are ongoing.

Meta-analysis for quantitative responses of soybean sudden death syndrome

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Soybean sudden death syndrome (SDS) is a soil-borne disease that causes annual yield losses up to \$669 million dollars. Decades worth of effort in mapping SDS resistance has pointed out a quantitative interaction between soybean and the casual pathogen *Fusarium virguliforme* in the United States. Inconsistencies in screening methodologies and naming system for quantitative trait loci (QTL) has resulted in confusion about the validity of each QTL in SDS resistance. More than 50 QTL are reported in the literature, and with the recent addition of single nucleotide polymorphisms (SNPs), reproducibility has become questionable especially because SDS occurrence is highly variable and depends on environment. Accordingly, this study aims to integrate and compile mapping results for SDS. A schematic ideogram was generated for direct visualization of resistance QTLs, and only eight regions were rediscovered with at least three citations. We propose that these regions be named as meta-Rfv (Resistance to *F. virguliforme*). Two regions of particular interests are the meta-Rfv18-01, which received the most literature support and co-localized with a soybean cyst nematode (SCN) resistance locus (*Rhg1*), and the meta-Rfv06-01 which is a region identified in studies using phytotoxic culture filtrate as well as historical SDS fields for resistance screening. Future studies on these two regions may illuminate the synergistic effects between SDS and SCN, and the genetics of soybean foliar resistance to SDS phytotoxins. In summary, meta-analysis simplifies the current status of SDS resistance mapping, and the open information will be useful for future research and breeding programs.

Novel alleles for Goss' wilt resistance to maize

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Since its discovery in 1969, Goss' wilt and blight, a vascular disease caused by the gram-positive bacteria *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), has emerged as one of the top four diseases of maize in the United States and Ontario, Canada (Mueller et al., 2016). In 2015 an estimated 139.8 million bushels of maize were lost due to this pathogen (Mueller et al., 2016). No source of complete resistance has been described for Goss' wilt, and little is known about the genetics and mechanisms of host resistance to Cmn. Our research utilizes the 282-line Goodman maize diversity panel, which has been used extensively to map quantitative traits (Flint-

Garcia et al., 2005). We have evaluated this population for Goss' wilt and will use genome-wide association mapping to identify candidate genes. In parallel, a population of disease resistance introgression lines (DRILs; Oh7B x NC344) has been evaluated. This population includes 265 unique lines with introgressions that tile the maize genome and will be used to map and characterize regions underlying Goss' wilt resistance. Preliminary findings have revealed a novel resistance QTL, and trials in 2017 will serve to confirm these findings.

Wound healing and disease resistance in potatoes

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Wounding and damage to potato tubers during harvest and handling provides entry points for infection by tuber rotting pathogens such as *Fusarium* spp. and *Pectobacterium* spp. Thus, suberization (healing) of wounds is an important part of protecting tubers against these pathogens. Because cell wall degrading enzymes (CDE) are important factors in disease caused by these pathogens and these CDE cannot degrade suberized tissues, this research examines the potential use of these CDE to determine the rate of suberization in wounded potato tuber disks. Within 3–5 days of wounding, the upper layer of tissue became resistant to degradation by pectinases and cellulases. This approach demonstrated that the Manistee variety developed a suberized barrier more quickly (3 days) than the Lamoka variety (~5 days). The Manistee variety developed suberization-related resistance to *F. sambucinum* at 12 days and *P. carotovorum* subsp. *carotovorum* at ~3 days. The Lamoka variety developed slower suberization-related resistance to *F. sambucinum* (14 days) and *P. carotova* (10 days). These results suggest that CDE may be useful in monitoring wound induced suberization, and these results provide further information on how suberized barriers may protect potato tubers against infection. The chemical changes in the suberizing tissues that are related to increased disease and enzyme resistance are now being investigated.

Genomic alterations in *Sclerotinia sclerotiorum* after sublethal exposure to a mitosis-inhibiting fungicide

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Sublethal exposure to antifungal drugs is known to cause genomic instability and emergence of genetic variation in human fungal pathogens. This may confer antifungal resistance or other adaptive traits. Sublethal fungicides may exert similar genomic stress on fungal phytopathogens, but this phenomenon has not yet been fully characterized. Five isolates of *Sclerotinia sclerotiorum* were exposed to sublethal doses of commercially formulated thiophanate-methyl (MBC; mitosis inhibition) for twelve consecutive generations with experimental replication. Seven of the ten exposed individuals showed decreased sensitivity. Whole-genome sequence data were obtained for pre- and post-exposure individuals and filtered for read depth and mapping quality. Characterization of 474 variants showed 317 indels and 157 SNPs with 69 variants in the genic and 405 in the intergenic regions. All genic mutations were in genes coding hypothetical proteins. No mutations were found in β -tubulin gene (target of MBC fungicides). Sublethal exposure to thiophanate-methyl generated variants in non-target genomic segments, some of which conferred decreased sensitivity. This is the first study to characterize genomic alterations in a plant pathogen after exposure to a sublethal fungicide. Future analysis will determine if sublethal fungicide stress leads to random or directional mutations.

The Dpp and Opp peptide ABC transporter systems are responsible for kasugamycin susceptibility in *Erwinia amylovora*

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Extensive use of antibiotic streptomycin to control fire blight disease, caused by *Erwinia amylovora*, leads to the development of streptomycin-resistant strains in the United States. It is thus recommended that kasugamycin be considered as an alternative to control fire blight disease. Therefore, it is critical to examine the molecular mechanisms underlying kasugamycin susceptibility in *E. amylovora*. In this study, the role of the Dpp and Opp peptide ABC transporter systems in conferring susceptibility to kasugamycin and blasticidin S in *E. amylovora* was investigated. Minimum inhibitory concentration (MIC) and spot dilution assays showed that deletion mutants of *dpp* enhanced resistance to kasugamycin alone, whereas deletion mutants of *opp* led to resistance to both kasugamycin and blasticidin S. In addition, bioinformatic analysis combined with qRT-PCR showed that the Rcs system negatively regulates the expression of *opp* operon. Electrophoretic motility shift assay further confirmed the direct interaction between the promoter of *oppA* and RcsA/RcsB proteins. However, neither Dpp nor Opp system contributed to disease progress on immature pears, hypersensitive response on tobacco leaves, and motility. These results suggested that both Dpp and Opp systems are responsible for the susceptibility of *E. amylovora* to kasugamycin.

Characterizing the diversity of pathogen virulence and host resistance for more effective management of *Phytophthora* root rot of soybean

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Disease management is more effective if the diversity of the pathogen present in an area is taken into account. *Phytophthora* root rot of soybean (PRR) is caused by at least two diverse oomycete species in the genus *Phytophthora*. Management measures for PRR include the use of resistant cultivars and seed treatment. The objective of this research was to characterize the virulence of *Phytophthora* spp. causing PRR in Illinois and to determine the repertoire of resistance genes available in public soybean cultivars. Soil samples were

procured from 26 counties in IL. Isolates were recovered from the collected soils using a soil baiting protocol. After recovery, *Phytophthora* isolates were placed in long-term storage and will be genotyped and phenotyped to characterize their genetic diversity. To assess the availability of major resistance genes and the levels of partial resistance, greenhouse experiments were conducted on 30 public soybean lines. The hypocotyl inoculation technique was conducted to assess race-specific resistance, and a layer test to evaluate partial resistance. Race specific resistance was determined with isolates with known virulence. Eleven cultivars had no major resistance gene or the defeated Rps7, while eight, four, and three cultivars carry Rps1a, Rps1k, and Rps1c respectively. Four more cultivars appear to carry a combination of two or more resistance genes. Evaluation of partial resistance on these cultivars is underway. Five cultivars with different resistance genes are now being tested in field experiments for management of PRR in combination with seed treatments.

Phenotyping soybean charcoal rot resistance for nested-association and genome-wide association mapping

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Soybean charcoal rot is caused by the fungal pathogen *Macrophomina phaseolina*. Resistance to charcoal rot has been challenging to exploit because of the lack of sources with high levels of resistance. To find new sources of resistance, two soybean panels were grown and inoculated with *M. phaseolina* using the cut-stem assay. The SoyNAM parents, consisting of 40 elite and diverse lines and of IA3023 as the recurrent parent, made up one panel. Plant introductions, consisting of 350 wild, landrace and elite lines representing a collection based on over 10 million high-quality single nucleotide polymorphic markers, made up the other panel. Seedling death rates and stem lesion lengths were collected for 15 days after inoculation. Both tests provided wide disease response ranges, and therefore show potential for discovering new sources of charcoal rot resistance and for association mapping studies. In the SoyNAM study, parent lines Skylla, LD02-9050, and PI 507681B were selected as candidates for nested-association mapping. Phenotypic data of the plant introduction panel is in progress and will be used in the genome-wide association mapping study.

Seedling resistance to stripe rust (*Puccinia striiformis* f.sp. *tritici*) in the North Dakota wheat nursery

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Stripe rust is an economically important disease of wheat caused by the obligate pathogen *Puccinia striiformis* f.sp. *tritici* (*PSTv*). Stripe rust incidence has increased in the Northern Great Plains and is likely attributed to the prevalence of a more aggressive *PSTv* population that is adapted to warmer climates. In 2016, stripe rust was documented on May 23 in southeast and southwest North Dakota (ND), which to our knowledge, was the earliest observation on record for the state. The early observation of stripe rust increased the risk for potential yield loss in susceptible spring wheat varieties. The objective of this research was to screen the ND wheat nursery (breeding lines and released wheat cultivars) for seedling (all-stage) resistance. Three single pustule isolates coding to races *PSTv*-37, *PSTv*-41, and *PSTv*-52 were used in a greenhouse screening of the ND wheat nursery. A few of the nursery lines conferred seedling resistance to *PSTv*-37. None of the lines conferred consistent seedling resistance to *Pstv* 41, yet some segregating populations expressed resistant infection types. Most commonly grown hard red spring wheat varieties in ND were either moderately susceptible or susceptible to the screened races. The high level of susceptibility in hard red spring wheat suggests additional information is needed on varietal susceptibility.

Investigating the interactions of the soybean cyst nematode with cover crops under greenhouse conditions

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Cover crops are being grown on an increasing agricultural land area in the United States annually, and there is great interest in learning how the soybean cyst nematode (SCN), *Heterodera glycines*, interacts with these plants. To assess the potential for cover crops to function as trap crops, roots from plants were grown in SCN-infested soil for 20 days and observed for SCN penetration. There were significantly more ($P < 0.05$) nematodes in crimson clover and mustard roots than in annual ryegrass and cereal rye roots, with cereal rye having significantly the fewest nematodes. Another experiment was conducted for 30 and 60 days to determine if cover crops directly decrease the population density of SCN. Annual ryegrass, radish, and cover crop mixes had significantly lower ($P < 0.05$) final:initial egg population density ratios compared to soybean, tomato, and fallow. Using soil left over from each experimental unit of the previous study, a 30-day experiment was conducted with a susceptible soybean cultivar to assess SCN reproduction in soils in which cover crops had been grown. There were significantly fewer ($P < 0.05$) SCN females on soybean following annual ryegrass compared to those following cereal rye, radish, tomato, and fallow. Results indicate that there is a potential for some cover crop species to negatively impact SCN population densities; however, additional experiments are needed to make broader inferences and to distinguish differences among cultivars.

Alternative hosts of *Xanthomonas vasicola* causing bacterial leaf streak of corn

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The pathogen *Xanthomonas vasicola* causes bacterial leaf streak of corn and was reported in the U.S. in 2016 corn samples from Nebraska fields. Because numerous monocotyledonous hosts have already been reported for *X. vasicola*, a greenhouse study was

conducted and repeated to test other potential hosts that are present in the U.S. Seed from 40 species, including crops, turf, native grasses, and weeds were sown in pots. *X. vasicola* suspended in water at 1×10^8 cfu/ml was sprayed onto plants and kept 12 hr in a humidity chamber at 100% relative humidity before returning them to the greenhouse. Plants were monitored for symptom development and disease severity was visually estimated. Disease severity varied by species from zero to moderately severe and symptomatic leaves were evaluated microscopically for bacterial streaming. Nine plant species developed disease in this experiment including oat (*Avena sativa* ‘Jerry’), orchardgrass (*Dactylis glomerata* ‘Latar’), Indiangrass (*Sorghastrum nutans* ‘Holt’), big bluestem (*Andropogon gerardii* ‘Champ’), little bluestem (*Schizachyrium scoparium* ‘Blaze’), timothy (*Phleum pratense* ‘Climax’), green foxtail (*Setaria viridis*), bristly foxtail (*S. verticillata*), and yellow nutsedge (*Cyperus esculentus*) and bacterial streaming was confirmed. These results are important as they may indicate additional plants that could become sources of inoculum or sites for population maintenance for disease outbreaks.

Distribution of bacterial leaf streak of corn caused by *Xanthomonas vasicola* in Nebraska

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Xanthomonas vasicola has been reported on a wide range of hosts around the world, and is most noted for causing gumming disease of sugarcane. The pathogen also causes bacterial leaf streak on corn in South Africa, and was reported in the United States in Nebraska field corn in 2016. A survey was initiated during the 2016 growing season to determine the distribution of *X. vasicola* on corn in Nebraska, as well as to identify agricultural practices that may be conducive to disease development. Surveys were distributed to farmers, crop consultants, Nebraska Extension personnel and other agribusiness cooperators. In addition to answering questions about cropping practices, respondents were asked to submit symptomatic leaf samples for pathogen isolation and confirmation. From the more than 2,000 surveys distributed, 409 were returned with samples. PCR assays were conducted on bacterial colonies isolated from symptomatic leaf samples, and results confirmed that *X. vasicola* was present in at least 51 Nebraska counties from the southwest corner to the northeast and through most of the counties in the central part of the state. None of the few samples received from the western or eastern most counties tested positive for the pathogen. Survey responses are also being analyzed to identify crop production practices that may have impacted disease development.

Effects of barley yellow dwarf virus on hard red spring wheat and spring durum

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Barley yellow dwarf (BYD) is an important viral disease of small grains in North Dakota (ND) and throughout the world. Several strains of *Barley yellow dwarf virus* (BYDV) and *Cereal yellow dwarf virus* (CYDV) cause BYD. These include BYDV-PAV and CYDV-RPV, which are routinely detected in ND. In recent years, the arrival of the cereal aphid vector in North Dakota has occurred earlier; increasing the risk for potential yield loss by BYD. The objective of this research is to assess plant loss and yield loss in hard red spring wheat and spring durum when inoculated with BYDV-PAV. Greenhouse and field trials were established to estimate yield loss, reduction of spikes, and reduction of dry shoot mass. Both greenhouse and field trials were designed in a randomized complete block with a split plot arrangement. Variety served as a main plot and inoculation served as a sub-plot. Yield loss was assessed in both greenhouse and field trials, whereas dry shoot mass reduction and spike reduction were assessed only in the greenhouse. Greenhouse trials indicated BYD resulted in yield losses of 26% and 25% for hard red spring wheat and durum, respectively. When inoculated, dry shoot mass was significantly reduced by 14.4% in hard red spring wheat. Field trials indicated yield losses of 10% for spring wheat and 13% for spring durum. The information from the research will be used to assess BYD risk and potential yield loss ramifications.

Effects of high relative humidity on severity of bacterial leaf streak of corn caused by *Xanthomonas vasicola* in the greenhouse

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Xanthomonas vasicola was confirmed for the first time in the United States in 2016 causing bacterial leaf streak on samples from Nebraska corn fields. The pathogen historically is most notable for causing gumming disease of sugarcane around the world, but had only previously been reported on corn in South Africa. The disease has since been reported in several additional states, however limited research has been conducted on weather conditions that favor disease development. To better understand the relationship between relative humidity (RH) and disease severity, corn plants were grown in a greenhouse and inoculated with *X. vasicola* in a humidity chamber. Plants were inoculated with 1×10^8 cfu/ml bacteria suspended in water and subjected to 100% RH treatments for 0, 4, 8, or 12 hr before moving plants to the greenhouse. Disease severity was estimated at 7, 10, and 19 days after inoculation as a percent of the total V4 leaf area. Disease severity generally increased with increased time spent at 100% RH. Disease also developed after 0 hr of 100% RH, suggesting that the pathogen does not require incubation at 100% RH to infect susceptible plants. Results will be used to develop inoculation protocols for experiments conducted in the greenhouse or field.

Minimal region required for the suppression of RNA silencing by *Wheat streak mosaic virus P1*

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Wheat streak mosaic virus (WSMV) is the most economically important wheat virus in the Great Plains of the USA. WSMV is the type species of the genus *Tritimovirus*, family *Potyviridae*, and is transmitted by the wheat curl mites, *Aceria tosichella* Keifer. Previously, we reported that WSMV P1 functions as the viral suppressor of RNA silencing (VSR) and pathogenicity enhancer. In this study, we examined the minimal region of P1 required for VSR function. Twenty-one deletion mutants of WSMV P1 were generated by progressively deleting from the N- and C-terminal regions. GFP reporter assay by agroinfiltration of deletion mutants revealed that deletion of up to 23 amino acids at the N-terminal region retained VSR activity. In contrast, even a single amino acid at the C-terminus is indispensable for VSR activity, suggesting that the C-terminal region of P1 is crucial for suppression of RNA silencing function. A single GW motif is present towards the C-terminus of P1, which is homologous to the AGO-binding linear-peptide motif conserved across metazoans and plants. Site-directed mutagenesis of the GW motif through a single point W303A mutation resulted in complete abolition of both VSR activity and pathogenesis, suggesting that WSMV P1 suppresses RNA silencing via AGO-binding.

Octapartite negative-sense RNA genome of *Wheat mosaic emaravirus* (WMoV) encodes dual suppressors of RNA silencing

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RNA silencing is the most effective antiviral defense mechanism in higher plants. However, viruses have evolved a variety of efficient counter-defense mechanisms by suppression of RNA silencing (VSR) to impede host defenses. The genus *Emaravirus* is comprised of members with single-stranded multipartite RNA genomes of negative orientation. No information is available on suppressors of RNA silencing encoded by the members of *Emaravirus* genus. *Wheat mosaic virus* (WMoV) is a distinct member of the genus *Emaravirus* and is transmitted by the wheat curl mite (*Aceria tosichella* Keifer). WMoV, the causative virus of High Plains disease, is an economically important virus in wheat and maize. In this study, we screened WMoV genomic RNAs 2-8 for potential ORFs encoding for VSR function. A GFP-reporter agroinfiltration assay demonstrated that proteins encoded by ORFs 7 (p7) and 8 (p8) suppressed silencing induced by single- or double-stranded RNAs (ss/ds RNAs), and efficiently disrupted the systemic spread of silencing inducers. Additionally, we examined the VSR function of p7 and p8 of WMoV by replacing the suppressor of RNA silencing (P1) of *Wheat streak mosaic virus* (WSMV), a member of the family *Potyviridae*, with that of p7 or p8. WSMVDP1-GFP with p7 or p8 of WMoV facilitated efficient cell-to-cell and long-distance movement of WSMV, apparently by rescuing the VSR activity of P1 with that of p7 or p8, thereby confirming p7 and p8 of WMoV as suppressors of RNA silencing. Chimeric expression of WMoV ORF 7 or 8 through *Potato virus X* (PVX) significantly increased pathogenicity of PVX in *Nicotiana benthamiana*. Conclusively, these data suggest that WMoV encodes dual suppressors of RNA silencing proteins to overcome the host defense mechanisms.

Varietal screening of wheat crop against root-lesion nematode in North Dakota

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The importance of root-lesion nematode (RLN; *Pratylenchus* spp.) on wheat production in the U.S.A. was demonstrated a decade ago in the Pacific Northwest but no such study was carried out in North Dakota (ND). In 2014 and 2015, nematode surveys in ND led to detection of *Pratylenchus neglectus* on wheat for the first time. Growing resistant and tolerant varieties is considered to be the best approach for managing RLN. To identify wheat varieties with resistance to *P. neglectus*, 15 hard red spring wheat and five durum wheat varieties were screened in clay loam soil infested with *P. neglectus*. Two resistant and two susceptible wheat entries were included as controls for comparison. Reproduction factors (RF) were determined by measuring the final and initial nematode populations. The varieties evaluated showed a range of RF values from 0.5 to 5.4 for the first experiment, and 0.1 to 4.1 for the second experiment. For both experiments, statistical differences were observed among the varieties. Qualitative classification of resistant, moderately resistant, moderately susceptible and susceptible was assigned to each variety based on RF. None of varieties were consistently classified as resistant, and the durum variety Mountrail was consistently classified as susceptible. More experiments will be conducted to confirm the resistance level of each variety. This study can assist farmers in varietal selection and protect their wheat crop from yield loss in RLN infested fields.

Elevated cyclic di-GMP levels confer an auto-aggregation phenotype in *Erwinia amylovora*

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Cyclic-di-GMP (c-di-GMP) is a ubiquitous bacterial second messenger that enables the transition from a mobile lifestyle to an attached lifestyle in the form of biofilms. Biofilm formation enables the fire blight pathogen *Erwinia amylovora* to attach and proliferate to a very high population size within xylem vessels. In order to study the effects of elevated intracellular c-di-GMP levels in *E. amylovora*, we constructed single, double and triple deletion mutants of phosphodiesterase enzyme encoding genes (*pdeA*, *pdeB* and *pdeC*) that are responsible for the degradation of c-di-GMP. We observed significantly elevated intracellular levels of c-di-GMP in the double deletion mutants, with the highest levels observed in mutants $\Delta pdeA/C$ and $\Delta pdeA/B/C$. Interestingly, these mutants also displayed a strong auto-aggregation phenotype when grown in liquid medium, and biofilm formation was negatively affected. Also, virulence was significantly reduced in these mutants. In order to determine the regulatory mechanism behind the auto aggregation phenotype, we screened a

transposon mutant library using the *ΔpdeA/B/C* mutant, for suppressors of the auto-aggregation phenotype. To date, we have screened 4500 mutants and have identified five genes that might potentially regulate auto-aggregation in *E. amylovora*.

Cross-pathogenicity of *Rhizoctonia* spp. isolated from multiple hosts to corn, soybean, and wheat

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Rhizoctonia spp. are important soil-borne pathogens with a wide host range causing damping off and stem and root rots in row crops. Often, isolates are tested for pathogenicity only on the host from which they were isolated but cross-pathogenicity may have implications on crop rotation. Our objective was to determine if isolates collected from multiple hosts in Nebraska are cross-pathogenic to corn, soybean, and wheat, which are common in a crop rotation in the region. *Rhizoctonia* isolates were recovered through a state-wide survey of 44 fields in 22 Nebraska counties in 2015 and 2016. Isolates were identified by sequencing the ITS regions. Pathogenicity test was conducted by inoculating 77 isolates onto corn, soybean, and wheat using rolled towel assay method. The 26 most virulent strains on the three hosts were used for greenhouse assay to confirm the virulence and each experiment was repeated once. Disease severity was assessed based on disease index scale 1-9 for soybean and 1-8 for corn and wheat. The 26 strains showed cross-pathogenicity on corn, soybean, and wheat in the greenhouse but cumulatively they were more aggressive on soybean. The 77 strains identified were recovered from multiple hosts as follows - chickpea (4), corn (25), dry bean (23), soybean (16), wheat (3), and grasses (6). The 26 isolates that were cross-pathogenic included *Rhizoctonia zeae* (10), *Rhizoctonia solani* AG-5 (7), AG-4 HG-II (4), AG-3 (1), binucleate *Rhizoctonia* AG-F (2), AG-K (1), and AG-L (1). Results suggest that *Rhizoctonia* spp. with cross-pathogenicity on corn, soybean, and wheat are common.

Co-inoculation of *Pythium sylvaticum* and *Fusarium oxysporum* on soybean seedling disease development

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Seedling disease of soybean is caused by *Pythium* spp., *Fusarium* spp., *Phytophthora sojae*, and *Rhizoctonia solani*. Multiple pathogens are frequently recovered together from diseased soybean seedlings, suggesting a seedling disease complex. *Pythium sylvaticum* and *Fusarium oxysporum* are frequently associated with seedling disease of soybean. A cup assay was used to evaluate the effect of the interaction between *Pythium sylvaticum* and *Fusarium oxysporum* species on soybean seedling disease development. Cups were inoculated with either *Pythium* alone, *Fusarium* alone, or co-inoculated with both genera. Non-inoculated cups were used as a control. Cups were sampled at 2, 4, 6, 8, and 10 days after planting (DAP) and seedlings were evaluated for disease severity (DS), wet root weight (WRW), and emergence (EME). Isolations on selective media were done to determine pathogen prevalence. Seedling disease was more severe in cups inoculated with *Pythium* compared to those inoculated with *Fusarium*. Generally, there was little difference in disease development between treatments inoculated with *Pythium* alone and those co-inoculated with *Pythium* and *Fusarium*. Over time, an increase in DS and WRW was observed in *Pythium*+*Fusarium* treatments and there was no effect of time on EME. Under the conditions used in these experiments, there was no observed interaction of *Pythium* and *Fusarium* on soybean.

Characterization of response to *Rhizoctonia solani* among soybean cultivars in field and greenhouse environments

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Rhizoctonia root rot of soybean caused by *Rhizoctonia solani* can be a major problem in Minnesota. This disease kills and stunts plants in early vegetative growth stages, especially when planting is delayed until late May due to wet soil conditions. There are no soybean cultivars marketed with clear resistance to this disease. The central goal of this project is to determine if early maturity group soybean germplasms vary in response to this disease and to identify those with different levels of susceptibility. Replicated field and greenhouse studies were conducted to determine if randomly chosen soybean cultivars respond differently to *R. solani* in growth or yield. Soil in field and greenhouse studies was infested with one isolate of *R. solani* AG2-2. Subsequent ratings were taken for stand count, disease severity, plant height, biomass, and/or seed yield. Cultivars responded with significant differences in plant height and stand count in the greenhouse and with significant differences in stand count and yield in the field for inoculated vs. noninoculated treatments. These initial studies suggest that northern soybean cultivars can respond differently to *Rhizoctonia* root rot which could relate to yield in the field under conditions favorable for this disease. Additional studies are underway to validate these results under varying conditions.

First report of *Setomelanomma holmii* on blue spruce branches exhibiting needle drop in Indiana

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Sudden needle drop of blue spruce (*Picea pungens*) (often referred to as SNEED) has been reported to be associated with the fungus *Setomelanomma holmii*. Symptoms include brown to purple needles which prematurely abscise from the tree, often leaving barren branches. Numerous samples of spruce branches are submitted annually to the Plant and Pest Diagnostic Lab at Purdue University for diagnosis of needle discoloration and defoliation. While *Rhizosphaera* and *Stigmina* spp. are frequently found to be associated with branches exhibiting needle drop, often in the absence of these fungi, small, black, globose, setose pseudothecia, measuring 133 to 300 µm (mean 190 µm) in diameter are observed on branch samples. Blastn analysis from a pure culture resulted in a 99.8% homology to

Setomelanomma holmii. While *S. holmii* was first observed in the United States in 1998 and has been confirmed morphologically within various states in the Midwest as well as Indiana in 2011, this is the first molecular confirmation and morphological description of *S. holmii* on blue spruce in Indiana.

Detection and quantification of *Septoria glycines* in soybeans with Real-time PCR

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Septoria glycines, the causal agent of soybean brown spot, is a prevalent foliar fungal pathogen in Illinois. The disease usually remains at the lower canopy and develops upward at maturity stage, which might lead to premature defoliation. In average, the yield losses caused by *S. glycines* are estimated to be 3 to 8% per year. Application of fungicides at the R3 stage is the most common method to control this disease. However, it is unknown if the fungicide applications are economically justified. Development of economic thresholds for fungicide application requires of an accurate quantification of the pathogen which is difficult with an organism that has a prolonged incubation period. To accurately track the development of the pathogen, quantitative real-time PCR (qPCR) primers and hydrolysis probes were designed from polymorphic regions of the actin, β -tubulin and calmodulin genes. Twenty-six *S. glycines*, two *Colletotrichum gloeosporioides* and four *Cercospora sojina* were used to test the specificity and sensitivity of the designed primers. The DNA was extracted by the CTAB method. Primers designed from β -tubulin and actin were specific for detecting *S. glycines*. No fluorescent signals were detected ($C_t > 35$) from other tested pathogens and soybean DNA. The qPCR technique developed in this research will be applied to developing a yield model in the further studies, which is indispensable to define an economic threshold for the application of control measures against chronic soybean diseases.

Virulence of *Rhizoctonia solani* AG2-2 isolates on sugar beet (*Beta vulgaris*) in response to low temperature

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Rhizoctonia solani AG2-2 is not only the causal agent of Rhizoctonia root and crown rot in sugar beet (*Beta vulgaris*) but it can also cause a seedling damping-off. Significant losses can occur in all regions where sugar beets are grown. One recommendation for managing seedling losses to *R. solani* is to plant early when the soil is still cool and the risk of infection is reduced. However, there are varying reports regarding what temperature presents a reduced risk with the most widely cited temperature below which *R. solani* becomes inactive being 15°C (60°F). In the current study, we evaluate the virulence of 35 *R. solani* AG2-2 isolates on sugar beet at 11°C and determine how virulence relates to the phylogenetic groups identified by Martin *et al.* (2014). Twenty-seven isolates had disease severity scores significantly higher than the controls when tested at 11°C and 16% of the inoculated plants were rated as dead at the end of the experiment indicating that even at 11°C, considerable stand losses are possible. Isolates in phylogenetic Group 2A were, on average, more aggressive than isolates in Group 2B at 11°C. While early season planting does provide some protection against damping-off, our data indicates that growers should not consider early planting to provide 'no risk' of *R. solani* infection. The risk in any particular field will depend on the specific isolates present as well as soil temperature, moisture and amount of inoculum.

Fungicide sensitivity of *Sclerotinia sclerotiorum* isolates from Brazil, Argentina, and the U.S.A.

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Sclerotinia sclerotiorum is a necrotrophic fungal pathogen wherein well-timed fungicide applications is a primary method of disease control. Fungicides such as boscalid, fluazinam, fluopyran, iprodione, procymidone, prothioconazole, pyraclostrobin, and thiophanate methyl are used for disease control in Brazil, Argentina and the USA. Sensitivity to these fungicides was determined for 120 *S. sclerotiorum* isolates from soybean and dry bean in Brazil, Argentina and the USA. Growth of 23 isolates at five concentrations of each fungicide in PDA was used to estimate a baseline EC₅₀. The average EC₅₀ plus two times the standard deviation was used as a single discriminatory dose to characterize relative sensitivity and EC₅₀ was determined for the 12 most tolerant isolates. One isolate from Brazil had increased tolerance to both dicarboximides, procymidone and iprodione, with relative growth 60–200% more than the average of all other isolates. EC₅₀ on boscalid for the 12 most tolerant isolates was three-fold higher than the baseline EC₅₀ (resistance factor 5.39). Four isolates showed moderate tolerance to prothioconazole (resistance factor 3.07). Five of six isolates with greatest tolerance to prothioconazole were from Nebraska, where triazoles are registered. Overall, there is a low frequency of reduced sensitivity isolates, nevertheless, alternating fungicides with the same target sites, such as procymidone and iprodione, should be avoided.

Dry bean rust (*Uromyces appendiculatus*) survey in North Dakota and evaluation of dry bean advanced breeding lines

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North Dakota leads dry bean (*Phaseolus vulgaris* L.) production in the US. Dry beans contributed approximately \$246 million to the state's economy in 2016. *Uromyces appendiculatus* (Pers.:Pers) is an autoecious, macrocyclic, obligate, biotrophic fungus that causes rust of dry bean. Rust epidemics were common in the state for decades until the deployment of the *Ur-3* resistance gene in the 1990's. A new rust race (20-3) conferring virulence to *Ur-3* was identified in North Dakota in 2008. This study seeks to determine current races of *U. appendiculatus* in North Dakota and evaluate advanced breeding lines for resistant against isolates of the most prevalent races

identified. Dry bean samples displaying symptoms of rust were collected from 61 fields in 2015 and 2016, 62 single pustule isolates were obtained, and the standard differential set of 12 dry bean lines was used to determine pathogen races. Preliminary results indicate that over 80% of isolates evaluated were characterized as race 20-3, which overcomes known resistance genes *Ur-3*, *Ur-6*, and *Ur-7*. Other races identified had similar virulence patterns, but overcame only two of these three resistance genes. Resistance to race 20-3 was identified in advanced lines from the NDSU breeding program from several dry bean market classes. Resistance appears to be conferred by the *Ur-11* and *Ur-5* genes. This research will provide valuable information on sources of adapted germplasm to breed for rust resistance in the region.

Integrated management of blossom end rot of tomato in Faisalabad, Pakistan

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Tomato (*Solanum lycopersicum*), ranks second in its importance among solanaceous crops. However, it requires an integrated/balanced cultural management to endure environmental stresses like blossom end rot (BER). Blossom end rot is an emerging physiological disorder in Pakistan which is caused by inadequate availability of calcium at the blossom end tissues of tomatoes. Current study was aimed to manage BER using irrigation, minerals and a hormone during 2013-2015 study period under greenhouse conditions. During first year of experiment, three different irrigation levels (2, 4, 6 times a day) and three Ca concentrations (0.5, 1.0 and 1.5%) were evaluated. During the second year, foliar applications of Abscissic Acid (ABA) was made at fruit setting stage keeping irrigation at optimum level. Visual disease assessments including disease incidence (%) were made weekly after symptoms appearance on fruits. Abscissic acid at 0.05% concentration revealed minimum BER symptoms (11.3%) on tomato fruits followed by calcium chloride at 1.5% (15.10%) and irrigation (20.1%). However, Gibberellic acid being antagonistic to ABA, suppresses the supply of calcium to the fruits. Statistical analysis revealed that total number of fruits and height of plants weren't affected by treatments at different concentration. This study suggests that high marketable tomato yield can be attained by keeping tomato plants in optimum level of irrigation applying optimum level of irrigation, fertilizer and ABA. Further work is needed to evaluate the interaction of minerals in the rhizosphere and their synergistic effects on occurrence of BER.

Fungicide sensitivity of *Sclerotinia sclerotiorum* from soybean in the North Central United States

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White mold caused by *Sclerotinia sclerotiorum* can be a yield-limiting disease in the temperate North Central soybean production areas, with reported losses >10 million bushels per year. No soybean varieties are completely resistant to disease, which means fungicides are a primary method of disease control. Single-site fungicides are used to control *S. sclerotiorum* in soybean, which are at high risk of resistance. Resistance to benzimidazoles were reported in Brazil and China, and to dicarboximides in China. Little is known about fungicide sensitivity of *S. sclerotiorum* from the North Central states. The purpose of this study was to collect isolates of *S. sclerotiorum* from soybean fields throughout the region and assess sensitivity to the most commonly used fungicides. Collected in 2015 were 344 isolates from: NE (196 isolates; 13 counties), IA (47 isolates; 7 counties), MI (66 isolates; 7 counties), and WI (35 isolates; 4 counties). Collected in 2016 were 190 isolates from: IA (30 isolates; 2 counties) and MI (160 isolates; 1 county). Currently underway is assessment of fungicide sensitivity for Picoxystrobin, Boscalid, Prothioconazole and Thiophanate-methyl using a single discriminatory dose. Baseline sensitivity of *S. sclerotiorum* will be determined using isolates from non-fungicide exposed dry bean nurseries in MI and NE. Results of this work will provide new information on fungicide sensitivity of *S. sclerotiorum* from soybean in the North Central states.

Population genetic diversity of *Sclerotinia sclerotiorum* populations from Brazilian soybean

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Sclerotinia sclerotiorum populations typically display low genotypic diversity, yet few studies have compared populations from soybean in Brazil to those in Argentina or the U.S. This study sought to characterize the diversity and population structure of 95 *S. sclerotiorum* isolates collected from soybean fields in Brazil, Argentina, and the United States. Seventy-five isolates were collected from Brazil, five from Argentina, and 15 from Nebraska. Populations were defined based upon the state the isolates were collected from; there were six populations in Brazil, one in Argentina, and one in the US. Genotyping was performed using amplification of 11 simple sequence repeat loci and identified 84 multilocus genotypes. Populations were significantly different between states (AMOVA $p < 0.001$). Calculation of the index of association showed three populations from Brazil and one from the US had significant linkage disequilibrium ($p < 0.05$). Significant differences between populations may be due, in part, to sexual recombination inferred within the four South American populations with non-significant linkage. However, additional isolate collection and genotyping are needed. Future research should focus on increasing the sample size and/or make comparisons using a whole-genome sequencing approach.

Can canopy reflectance be used for early detection of soybean sudden death syndrome?

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Remote sensing can be used for early detection of plant diseases by objectively and nondestructively measuring changes in radiation reflected from plant canopies. We evaluated the usefulness of spectroscopic reflectance wavebands in the visible (440-690 nm) and near-infrared (NIR) (760-860 nm) regions for early detection of soybean sudden death syndrome (SDS). Canopy reflectance was measured weekly in 240 soybean field quadrats (1.5 m × 3 m) using a hand-held, multispectral radiometer between late June (34 DAP) and mid-September (123 DAP), in 2016. Visual assessments of SDS foliar severity and incidence were obtained weekly after symptoms appearance. Canopy reflectance at 460 nm to 810 nm were regressed against visual disease assessments. Disease incidence was divided into four ranges (0–5%, 6–30%, 31–60% and 61–100%) and plotted against canopy reflectance spectra to determine the wavebands that best discriminate between disease levels. Wavebands in the visible region detected SDS earlier (55 – 62 DAP) than in the NIR (67 DAP). Canopy reflectance measured at 460 nm, 510 nm, and 710 nm wavebands explained more variability in disease (49%, 48% and 48%, respectively), than NIR wavebands of 760 nm and 810 nm (15% and 18%, respectively). Reflectance spectrum plots revealed that wavebands in both the visible and NIR regions discriminated between disease levels. This study suggests that wavebands in the visible and NIR regions may be useful for early SDS detection in the field. Further work is needed in more locations and years.

Characterization of fungi and oomycetes causing root rot in *Phaseolus vulgaris* L. at Popayán, Colombia

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Common bean is an important crop for food security in Latin America and Africa for its nutritional value. However, the crop has production limiting factors including root diseases, which are a major concern. The aim of this study is to determine the fungal and oomycetes pathogens in a field at the CIAT Popayán Experimental Station where common bean breeding material is screened for resistance to root rot. This field site is known to have high disease pressure, but the pathogens are uncharacterized. The field plot has sixteen subplots, which were sown with the susceptible NUA-35 line. Root rot symptoms were recorded for each subplot, and a composite 50 ml subsample of soil per subplot was randomly collected from 15 points per subplot and stored at –80°C. Soil was collected 15 days before sowing, and 15 and 30 days after planting and repeated over two planting seasons, March and October of 2014. A total of 842 fungal and oomycete isolates were recovered by dilution plating the soil onto PDA medium. These isolates will be identified to species level and pathogenicity of the isolates will be determined. DNA was isolated from the soil samples and primers for metabarcoding analysis will be used for identification of the fungal and oomycete taxa, including non-culturable species.

Fungicide sensitivity of *Alternaria* spp. causing *Alternaria* leaf spot on sugarbeet in Michigan

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Alternaria leaf spot (ALS), caused by *Alternaria* spp., can occur wherever sugarbeet is grown. Infection by *Alternaria* spp. and disease management has historically been considered a minor issue in sugarbeet production in the US. Recently, increased incidence and severity of ALS has been observed in MI at levels high enough to have the potential to cause yield loss due to defoliation. The objectives of this monitoring program were to: 1. collect ALS-infected sugarbeet leaves from commercial production fields in eastern Michigan; and 2. determine the sensitivity of the populations of *Alternaria* spp. to fungicides in three classes, including: demethylase inhibitor fungicides [difenoconazole (DA), fenbuconazole (FA), flutriafol (FL), prothioconazole (PA), and tetraconazole (TA)]; quinone outside inhibitor fungicides (pyraclostrobin, PS); and organo-tins (tri-phenyl tin OH, TPTH). Leaves with symptoms of ALS were sampled from sugarbeet fields in east-central Michigan. Mono-conidial isolates were obtained to determine sensitivity to each fungicide above. A spiral gradient dilution method was used to determine and estimate the fungicide concentration which caused a 50% inhibition of fungal growth (EC₅₀) in vitro for all isolates. A total of 143, 140, 13, 131, 143, 147 and 144 isolates were screened against DA, FA, FL, PA, PS, TPTH, and TA respectively and the mean EC₅₀ values were 3.0, 70.9, 46.8, 52.4, 62.0, 16.4 and 87.4 for DA, FA, FL, PA, PS, TPTH, and TA respectively.

A gain-of-function mutation in a zinc-finger transcription factor confers multidrug resistance in *Sclerotinia homoeocarpa*

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Repeated applications of fungicides to control fungal diseases in human and plants have led to the emergence of multidrug resistance (MDR). An understanding of MDR mechanisms is crucial for combating fungal pathogens, but is understudied in plant pathogenic fungi. In this study, RNA-seq analysis of *Sclerotinia homoeocarpa*, the causal agent of dollar spot on turfgrasses, MDR and fungicide sensitive strains indicated that genes involved in xenobiotic detoxification, the cytochrome P450s (CYP450s) and ATP-binding cassette (ABC) efflux transporters, were overexpressed in a MDR strain. We provide evidence that this pathogenic fungus detoxifies xenobiotics through elaborate usage of CYP450s and ABC transporters, and overexpression of both genes families are involved in MDR. In addition, this study reveals that a Zn₂Cys₆ transcription factor (Shxdr1) regulates the expression of both CYP450s and ABC transporters, and that a dominant gain-of-function mutation in Shxdr1 is responsible for overexpression of the genes, resulting in resistance to many types of chemicals. Our mechanistic understanding of the MDR pathway in *S. homoeocarpa* may facilitate the development of new fungicides to control MDR populations in plant and human pathogenic fungi.

Integrating varietal resistance and fungicide timing to manage foliar diseases of spring barley

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North Dakota (ND) is one of the top three producing states of barley in the United States. Two spring barley types are grown in ND; two-row and six-row. Foliar diseases, such as net form net blotch, spot form net blotch, spot blotch and bacterial leaf streak, can limit yield and reduce barley quality in a given year. Thus, the integration of varietal selection and fungicides becomes instrumental in the management of foliar diseases. The objective of this study was to evaluate the role of host resistance and fungicide timing on the management of foliar disease in spring barley. Two trials were established in ND and were designed in a randomized complete block using a split-plot arrangement. Variety served as the main plot and fungicide timing as the sub-plot. Three, two-row spring barley varieties were used; Conlon, Pinnacle, and Genesis. Four fungicide timings were tested and included a non-treated control, single fungicide application at Feekes 2-3 (tillering), single fungicide application at Feekes 10.5 (full-head) and a sequential treatment at both Feekes 2-3 and Feekes 10.5. Disease developed at both locations and statistical differences were observed among varieties for flag leaf severity and yield. With some exceptions, treatments including a fungicide application at Feekes 10.5 resulted in lower year-end disease severity value. Results from this study will be used to update fungicide recommendations for barley growers in ND and the United States.

Population dynamics of *Erwinia amylovora* on apple flower stigmas and effect of antibiotic treatment

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Fire blight, a disease of pome fruit, is caused by the gram-negative bacterium *Erwinia amylovora*. If left unchecked, fire blight can decimate an orchard by not only destroying the current season's crop but lowering production in future seasons. The start of the epidemic occurs on flower stigmas where *E. amylovora* populations can attain populations sizes of 1×10^7 colony forming units (CFU). This upsurge of cells allows for disease to spread easily from flower to flower across an orchard by weather related events and insects. Currently, growers have three antibiotic options to control fire blight: streptomycin, kasugamycin, and oxytetracycline. Since populations rise over the course of the bloom period, timing of when antibiotics are applied may aid in control of fire blight. Cell buildup is also affected by environmental conditions occurring during bloom. This study asks if timing, both of antibiotic treatment along with weather factors, influences *E. amylovora* populations over the course of bloom. Stigmas of flowers open for one day were inoculated with $1 \times 10^{3-4}$ CFU *E. amylovora* which was preceded or followed by aerial application of each registered antibiotic or a water check. Flower were sampled every 24 hours for 5 days after inoculation to monitor *E. amylovora* populations. Hourly weather data collected during the 5-day study were analyzed for impacts on populations. From comparing trends between the 2016 and 2017 seasons, weather seemed to have an impact on antibiotic efficacy. There were differences in population responses to the timing of antibiotic application as well. Overall, this study will help growers use antibiotics along with weather forecasting programs to better suppress *E. amylovora* populations during bloom.

Plant-parasitic nematodes on field pea and their association with soil edaphic factors in North Dakota

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Plant-parasitic nematodes can cause reduction in plant height and yield on field pea. Nematode surveys were carried out in pea fields to investigate their prevalence, abundance, and association with soil properties in North Dakota. A total of 194 soil samples were collected from 12 counties during 2014 to 2016. Soil property analysis of 83 samples was performed to ascertain the association of nematode densities with soil factors (texture; organic matter; soil nutrient). Plant-parasitic nematodes, *Paratylenchus* (Absolute Frequency = 74%; Highest Density = 35,570/kg of soil) and *Tylenchorhynchus* (44%; 9,900) were the most frequent nematodes found in all surveys. Other nematodes were also detected in pea fields, including *Helicotylenchus* (Absolute Frequency = 18%; Highest Density = 3,695/kg of soil), *Xiphinema* (12%; 350), *Pratylenchus* (9%; 2,795), *Hoplolaimus* (3%; 530), *Meloidogyne* (1%; 100) and *Paratrichodorus* (< 1%; 110). There were good, negative correlations between densities of *Pratylenchus* and % clay ($r = -0.7$; $P < 0.05$) and between densities of *Tylenchorhynchus* and % clay ($r = -0.5$; $P < 0.001$). However, there was weak association between other nematodes and soil properties (% of sand, silt, clay and organic matter; soil nitrogen and potash). The nematode information obtained from this study would help determine the risk of nematode diseases in pea fields and design management strategies to minimize crop damage to improve pea production in North Dakota.

Efficacy of a seed treatment combination (oxathiapiprolin and metalaxyl) toward *Pythium* and *Phytophthora* spp. on soybean in field trials in Ohio

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Seedling diseases caused by *Pythium* and *Phytophthora* spp. are some of the most common disease problems in soybean in Ohio. Lumisena™ (oxathiapiprolin) is a new fungicide seed treatment assigned to FRAC group U15. The objective of this field trial was to determine the efficacy of a combination of oxathiapiprolin and metalaxyl seed treatment in field trials on seven (Clermont, Conrad,

Dennison, Kottman, Lorain, Sloan and Streeter) soybean cultivars with different levels of resistance towards *Pythium* and *P. sojae*. This study was conducted at four locations with a long history of stand establishment issues caused by both *Pythium* and *Phytophthora* spp. in Ohio during 2016. This study was a split plot design in a randomized complete block with 5 or 6 replicates and cultivars were the main factor and the seed treatments of oxathiapiprolin (24 µg a.i./seed) plus metalaxyl (0.13 oz a.i./cwt) or nontreated. Stand counts and yield data were collected from each plot and analyzed using PROC GLM and mean separation by Fisher's protected Least Significant Difference ($\alpha = 0.05$). There was a fungicide by cultivar interaction at 3 of the 4 locations. Yields of seed treated with fungicide were significantly higher than the nontreated for Conrad in all four locations; Clermont at three; as well as Sloan and Streeter at one. The combination of oxathiapiprolin and metalaxyl provided protection in fields with *Pythium* spp., *Phytophthora sansomeana* and *Phytophthora sojae*.

Investigating diversity of *Phytophthora sojae* within two Ohio fields

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Phytophthora root and stem rot of soybean, caused by *Phytophthora sojae*, has been a yield limiting disease since its discovery in Ohio during the early 1950's. Characterizing the level of diversity in *P. sojae* subpopulations is an ongoing effort to provide information about its population structure. The purpose of this study was to collect *P. sojae* isolates from two fields in northwest Ohio with a long history of disease, and to characterize the pathotypes as well as the genetic diversity. Ninety-six soil samples were collected in a grid pattern at the Northwest Agricultural Research Station near Hoytville, Ohio. Isolates of *P. sojae* were recovered by baiting the soil to encourage disease development on the moderately susceptible soybean cultivar, Sloan. Pathotyping of the 79 isolates using a set of 14 differentials identified 52 distinct pathotypes with a simple biodiversity index of 0.66 and a Shannon's diversity index of 3.71. The complexity of these isolates varied widely: 10, 19, 18, 14, and 13% of the isolates had a complexity of 1, 2, 3, 4, and 5, respectively. The highest level of complexity was observed for two isolates that were virulent on 12 of 14 differentials. Following pathotype determination, genetic diversity will be determined using a set of polymorphic simple sequence repeat (SSR) markers. We expect a low level of genetic diversity across the field due to the low level of outcrossing thought to occur in *P. sojae* populations. However, the pathotype results indicate that the diversity of *P. sojae* is highly variable for a homothallic species.

Sensitivity of oomycete species associated with corn to mefenoxam and ethaboxam

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Oomycetes cause significant damage to row crops worldwide. In Michigan, *Pythium* and *Phytophthora* species pose a threat to corn, causing root and stalk rot, stand reduction, and seed decay. Screening these species for fungicide sensitivity is useful for disease management. A high-throughput microtiter plate assay was used to evaluate fungicide sensitivity of 60 oomycete isolates consisting of 21 *Pythium* spp. and *Phytophthora sansomeana*, isolated from corn grown in Michigan. Isolates were grown on one-quarter strength potato carrot agar medium, macerated through a 10 ml syringe, and inoculated into a 96-well microtiter plate containing half-strength V8 broth amended with ethaboxam and mefenoxam at final concentrations of 0, 0.01, 0.1, 0.5, 1, 10, and 100 µg a.i. ml⁻¹. Optical density measurements were used to estimate EC₅₀ values. Results indicate 57 isolates had EC₅₀ < 1 µg ml⁻¹ to mefenoxam, and 3 isolates of *Pythium ultimum* var. *ultimum* were significantly less sensitive (EC₅₀ > 85 µg ml⁻¹). For ethaboxam, *Pythium* aff. *torulosum* and *Pythium rostratiformis* were found to be significantly less sensitive (EC₅₀ > 100 µg ml⁻¹), while 47 isolates had EC₅₀ < 1 µg ml⁻¹. Knowledge of fungicide sensitivity allows growers to develop effective disease management strategies to combat yield loss and fungicide resistance.

Characterization of genes in *Lysobacter enzymogenes* essential for inhibiting soybean fungal pathogens

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Lysobacter enzymogenes strain C3 (LeC3) is a potential biocontrol agent for plant diseases caused by fungi and oomycetes. In this study, a mutant library of LeC3 was generated and screened using soybean white mold pathogen (*Sclerotinia sclerotium*). Our results showed that 141 mutants exhibited significant decrease in inhibiting hyphae growth of *S. sclerotium*. Among them, 73 mutants no longer inhibited *S. sclerotium* hyphae growth and nine mutants were selected for further characterization. These nine mutants were partially recovered in their abilities in inhibiting hyphae growth of *S. sclerotium*. In addition, all nine mutants decreased significantly in their abilities in suppressing spore germination of the sudden death syndrome pathogen (*Fusarium virguliforme*) and in producing four extracellular enzymes. Furthermore, secretion of the heat stable antifungal factor (HSAF), a fungal specific antibiotic of LeC3, was significantly decreased in all nine mutants. Collectively, our findings suggest that the nine genes of LeC3 play important roles in antagonism against fungal pathogens probably by affecting both extracellular enzymes and HSAF.

Genetic analysis of host resistance to sorghum leaf blight identifies conserved disease resistance loci in the grasses

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Sorghum leaf blight (SLB) and northern corn leaf blight (NCLB) are important diseases of sorghum and corn, respectively. Both diseases are caused by the same pathogen, *Setosphaeria turcica*. Aside from grain loss, it can also increase susceptibility to other pathogens and decrease forage quality. Not only is this an important disease to study in order to decrease yield loss due to biotic stress, but it is an excellent system to study the evolution of pathogens on different hosts. Isolates are generally host-specific wherein maize isolates generally only cause disease on maize and sorghum isolates generally only cause disease on sorghum. While *S. turcica* causes disease both on maize and sorghum, we hypothesize that there are shared mechanisms of resistance to *S. turcica* in maize and sorghum. Host resistance to NCLB in maize has been extensively studied, but little is known about the genetics of resistance in sorghum. In this study, we evaluated the Sorghum Conversion Panel (n = 798) to identify regions of the sorghum genome associated with resistance to *S. turcica*. The panel was screened by inoculating field-grown plants with selected sorghum *S. turcica* strains. Disease severity was rated using a percentage scale three times after inoculation with 7 day intervals between the ratings, and the area under the disease progress curve was calculated. Using genome-wide association mapping, we identified 42 loci associated with SLB resistance. Among those loci, 4 candidate loci contained sorghum homologs of maize NCLB resistance genes.