2015 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in East Lansing, Michigan, June 10–12, 2015. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows: Acharya, J., Kasper, T., Moorman, T., Lenssen, A. W., and Robertson, A. R. 2016. Effect of cereal rye winter cover crop (*Secale cereal*) termination on corn seedling diseases. (Abstr.) Phytopathology 106(Suppl. 1):S1.1. <http://dx.doi.org/10.1094/PHYTO-106-1-S1.1>

Effect of cereal rye winter cover crop (*Secale cereal*) termination on corn seedling diseases

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Cover crops provide multiple benefits to farming systems, however, winter rye (*Secale cereal*), a common cover crop in Iowa occasionally reduces corn yield. Our hypothesis is that rye serves as a host for corn pathogens and provides a “green bridge” between the soybean and corn crops. Field studies were conducted to investigate the effect of rye cover crop termination timing on corn seedling disease and yield. Treatments were rye termination at 21, 14, 10, 3 days before planting (dbp) and 1 day after planting (dap) corn. Corn shoot height, dry shoot weight and radicle length were reduced ($P < 0.01$) following rye compared to no rye. Radicle rot incidence, radicle disease severity, and *Pythium* incidence were greater ($P < 0.05$), while the incidence of *Fusarium* recovered did not differ, in corn following rye compared to no rye. Radicle infection and *Pythium* incidence was reduced in treatments where rye was terminated 21, 14 and 10 dbp compared to 3 dbp, and 1 dap. *Fusarium* incidence was less in most treatments than where rye was terminated 1dap. Several *Pythium* species and *Fusarium* species were identified on corn roots following rye that were not found on corn that did not follow a rye cover crop. Corn yield was reduced when rye was terminated 3 dbp and 1 dap compared to 10 or 14 dbp or no rye. Understanding how rye termination date affects corn seedling disease and yield will enable improved management practices to ensure optimum use of cover crops in a corn farming system.

Variations in phylogenetic signal among genes for species delimitation in North American yellow morels

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Millions of pounds of morels (*Morchella* spp.) are harvested every spring, yet little is known about the diversity among this fungal genera. Previous analyses have identified three main *Morchella* clades: yellows (*M. esculenta*/Mes clade), blacks (*M. elata*/Mel clade) and grays (*M. rufobrunnea*/Mruf clade). However, look-a-like species within these clades are indistinguishable even with the use of the internal transcribed spacer (ITS) region. More informative genetic markers are needed to effectively resolve morel diversity. To address this need we used the genealogical concordance phylogenetic species recognition (GCPSR) criteria for three gene fragments: elongation factor 1-

alpha (EF-1 α), the second largest RNA polymerase subunits (RPB2), and nuclear ribosomal large subunit (LSU) 28s rDNA. This allowed us to identify the morphological and phylogenetic species diversity of morels collected in Indiana in 2013, and test which genes are most useful in species delimitation. GCPSR analyses resolved over 80% of our isolates to one lineage despite morphological variability, while Bayesian Inference of EF-1 α and RPB2 provide the most informative phylogenetic signal for North American morels in the Mes clade. Our results identify methods to effectively resolve *Morchella* species limits and diversity. Ultimately, identification of what we are harvesting and its impacts on fungal resources will allow for the development of management strategies to ensure a sustainable morel crop.

Impact of foliar fungicides on Gibberella ear rot and deoxynivalenol levels in corn

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Fusarium graminearum infects corn seedlings, stalks, and ears. The most important of these is the ear infection, which reduces yield and can result in accumulation of the mycotoxin deoxynivalenol (DON). Certain fungicides have been promoted to reduce Gibberella ear rot, however data on their efficacy and effect on DON is limited. Fungicide efficacy trials were established near Lafayette, IN in 2013 and near Wanatah, IN in 2013 and 2014. Three fungicides, pyraclostrobin (Headline), prothioconazole (Proline), and azoxystrobin + propiconazole (Quilt Xcel) were applied at silking (R1) according to manufacturer's recommended rates. Fungicide efficacy was tested under inoculated and non-inoculated conditions. All ears in each inoculated plot were injected with a *F. graminearum* macroconidia suspension, at a rate of 37,500 conidia per ear. Inoculation significantly increased disease severity and DON ($P < 0.001$, $P < 0.001$, respectively) in all years and locations. Disease severity ranged from 10.5% to 77% in inoculated plots and from 0% to 5.6% in non-inoculated plots. DON levels ranged from 12.5 ppm to 76.5 ppm in inoculated plots, and from 0.7 ppm to 9.7 ppm in non-inoculated plots. However, disease severity and DON were not significantly reduced by fungicide treatments in any year or location compared to the non-fungicide treated controls. Yield was also not significantly impacted by fungicide treatments. These results indicate that additional in-season control measures are needed to help manage Gibberella ear rot.

Genome wide association studies and genetic analysis of leaf rust resistance in durum wheat

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Leaf rust (*Puccinia triticina* Eriks.) is increasingly impacting durum wheat production since the detection of races with virulence to widely grown cultivars in many durum production areas worldwide. A highly virulent leaf

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rust race on durum wheat, BBBQ, was collected, over the past few years in California. This race has similar virulence to races previously identified in Mexico which have caused severe yield losses. Most of the currently grown durum wheat cultivars in the U.S. are susceptible to BBBQ race. The objective of the study is to identify sources of resistance to BBBQ, and other U.S. races at seedling stage alongside international virulent races at adult stage in field experiments. A core set of 497 accessions from the USDA-ARS National Small Grain collection were evaluated as seedlings in the greenhouse to four *P. triticina* races (BBBQ, BBBD, MBDS, and TBBJ) with virulence to locally adapted durum cultivars. These accessions were also evaluated at adult plant stage in field experiments in Mexico, USA, Ethiopia, and Morocco. Association mapping and biparental populations are being used to map the resistance loci and study their inheritance. Association mapping, based on the infection type to different leaf rust isolates at seedling stage in the greenhouse and at adult stage in the field revealed 107 significant SNP markers (P value < 0.001 and positive false discovery rate < 0.1), representing 48 different loci. A total of 23 markers were significantly associated with leaf rust response at seedling stage. At the adult stage, 84 markers were significant, of which 26 were located on chromosome 2B. The inheritance study on eight biparental populations showed that both recessive and dominant genes confer resistance to BBBQ race. Bulk segregant analysis is ongoing to determine the genetic locations associated with resistance. The findings will contribute to diversify leaf rust resistance in durum wheat breeding programs.

Winter rye cover crops as a host for corn seedling pathogens

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Phytopathology 106(Suppl. 1):S1.2

Cover cropping is an expanding conservation practice that offers substantial benefits to soil protection, soil health, water quality, and potentially crop yields. Presently, winter cereals are the most widely used cover crops in the upper Midwest. However, winter cereal cover crops preceding corn, may dampen beneficial rotation effects by putting two grass species in succession. Here, we show that cereal rye cover crops can host pathogens capable of causing corn seedling disease. We used qPCR to quantify densities of *Fusarium graminearum*, *Fusarium oxysporum*, *Pythium sylvaticum* and *Pythium torulosum* in roots of glyphosate terminated cereal rye cover crops from several field experiments. We found that corn seedling pathogens can increase in density on recently killed roots of cereal rye. *F. oxysporum* was detected in 96% of cereal rye root samples, at densities up to 201 pg DNA per mg dry root tissue. Corresponding values for *P. sylvaticum* were 78% and 620 pg per mg; for *P. torulosum*, 42% and 1.5 pg per mg; for *F. graminearum*, 25% and 0.8 pg per mg. We also tracked fungal and oomycete community succession in roots of cereal rye over time after termination using a non-targeted amplicon sequencing approach, and detected other potential shared pathogens between cereal rye and corn. This work suggests the potential for elevated disease pressure on corn seedlings following cereal rye cover crops, although this risk will be heavily influenced by environmental conditions.

Susceptibility to *Fusarium thapsinum* in grain sorghum involves host transcriptional reprogramming

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Fusarium thapsinum (FT) is a hemibiotrophic fungus causing stalk rot disease in sorghum. Thus far, identification of resistant lines through conventional means has been tedious. The objectives of this study were to: i) discover differentially expressed genes (DEG) of sorghum in response to FT inoculation and understand their contribution to resistance, and ii) investigate sorghum expression profile changes that may correspond to lifestyle transition of the fungus. Two sorghum genotypes resistant (SC599) and susceptible (Tx7000), were grown in the greenhouse and inoculated with FT. Control plants were mock-inoculated with phosphate-buffered saline. Sorghum stalk RNA was extracted from 3 biological replicates at 2, 7, and 30 d post-inoculation (dpi) and was subjected to RNA-Seq. Analysis for DEG was performed with DESeq2 and pathway analysis was performed to explore DEG. 4412 annotated genes were identified across the 3 post-inoculation time points (pti) as differentially expressed between 2 genotypes of which 1153 were components of 175 known metabolic pathways. 19 genes were common among the 3 pti. Most genes in the resistant host did not show a significant differential expression at 2 and 7 dpi, while those of the susceptible exhibited significant upregulation, demonstrating the involvement of “susceptibility genes” in disease manifestation. The opposite phenomenon was evident at 30 dpi, suggesting that host transcriptional reprogramming may coincide with the switch of FT from biotrophy to necrotrophy within the host.

Detection of Pea Seed-borne Mosaic Virus (PSbMV)

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The dry pea (*Pisum sativum* L.) growing regions in North Dakota and Montana account for 85% of US production. Pea Seed-borne Mosaic Virus (PSbMV), a potyvirus capable of reducing dry pea yield from 35 to 82% depending on virus pathotype and pea cultivar, recently has been detected in these important pea growing states. PSbMV is transmitted in seed and by aphid vectors. In 2013, PSbMV was confirmed in 30% of tested dry pea seed-lots. The reduction of contaminated seed and low levels of aphid vectors contributed to a reduction of PSbMV to approximately 12% of seed lots tested in 2014. ELISA typically is used for virus detection, however, RT-PCR has proven more sensitive to detect PSbMV. Unfortunately, RNA extractions required for RT-PCR are time consuming and costly. The objective of this research was to develop an immunocapture RT-PCR protocol to detect PSbMV directly from the ELISA plate, eliminating the need for RNA extractions. This method has proven to be as sensitive at detecting PSbMV in infected plant tissue as extracting RNA and performing RT-PCR. The use of immunocapture RT-PCR also has been shown to be more sensitive than ELISA, detecting the virus in 75% of PSbMV ELISA negative single lesions in *Chenopodium quinoa*. While the increased sensitivity may not be required for routine detection of PSbMV in seed lots, this PSbMV immunocapture RT-PCR assay is an important research tool for detecting low-titer infections in single-lesion or less-common hosts.

Effects of fluopyram on *Heterodera glycines* under greenhouse conditions

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Soybean cyst nematode (SCN, *Heterodera glycines*) is one of the most economically important soybean pathogens in the United States. Currently, best management practices include using resistant varieties and crop rotation with nonhosts. An increase in SCN populations virulent on PI 88788 requires development of new control methods, such as seed treatment nematicides. The objective of this study was to evaluate the effect of the fungicide fluopyram on SCN under greenhouse conditions. Treatments were Poncho/VOTiVO, Poncho/VOTiVO with 0.075 mg ai/seed fluopyram, 0.15 mg ai/seed fluopyram, and a nontreated control. All treatments received a base of 0.016 mg ai/seed penflufen, 0.016 mg ai/seed trifloxystrobin, 0.075 mg ai/seed prothioconazole, and 3.75 g ai/100 kg seed metalaxyl. Treatments were tested on resistant (Stine 26RD02) and susceptible (Stine 48RD00) soybean cultivars, replicated 6 times and collected data at 30 and 60 days. At 30 days both fluopyram treatments significantly decreased female densities and R_f ($p = 0.10$) on resistant and susceptible cultivars. At 60 days, all treatments decreased female and egg counts on the resistant cultivar compared to the check. On the susceptible cultivar only the higher rate of fluopyram decreased females and there were no differences among treatments in number of total eggs. Fluopyram decreased fecundity on the susceptible cultivar at 30 days, but no fecundity effects were observed on the resistant cultivar. Fluopyram treatments decreased dry root weights at 30 and 60 days and shoot weight at 30 days. Eggs/gram root was significantly lower on the resistant cultivar at 60 days and the susceptible cultivar at 30 days. These results show that fluopyram can reduce SCN females and eggs, however, the mode of action for the effect is not known. Combining treatments like fluopyram and SCN resistance could be a more effective means of managing SCN and needs further evaluation under field conditions.

Winter wheat grain yield gain from fungicide application is influenced by amount rainfall

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Winter wheat is susceptible to several fungal pathogens throughout the growing season. Fungicide application is an effective strategy in the management of fungal diseases, however, for fungicides to be profitable, significant fungal disease pressure and favorable weather conditions should be present. To determine if winter wheat yield response to fungicide application at flowering growth stage was related to the amount of rainfall, yield from fungicide treated plots was compared to non-treated plots for 21 to 30 hard red winter wheat cultivars planted at 9 location years. At all locations, Prosaro®/Folicur® fungicide was applied at flowering timing (Fekees 10.5.1) for the fungicide treated plots. Yield gain (difference between treated and non-

treated) was greatest at locations with higher rainfall. Yield gain had a positive and significant linear relationship with cumulative rainfall in May and June ($R^2 = 59\%$, $P = 0.0151$) indicating that a higher amount of accumulated rainfall in May and June increased chances of getting a higher yield gain from fungicide application. These results indicate that use of fungicides when there is sufficient moisture may increase chances of profitability.

2014 survey of corn foliar diseases in North Dakota

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The number of corn acres in North Dakota (ND) has nearly doubled since 2009, which has elevated concerns with disease-related problems. In particular, Extension personnel have received numerous questions regarding the economic benefits of applying foliar fungicides. Prior to 2014, the prevalence of diseases found in ND corn was relatively unknown. Therefore, a survey was needed to document foliar diseases of corn in ND. During September of 2014, when corn was at or beyond vegetative growth stage tasseling (VT), 62 fields were arbitrarily selected in the major corn growing regions of ND. At each field, a "W" pattern was used to survey 10 plants from 5 locations within a field. Foliar diseases were identified by visual symptoms. Four diseases of corn were identified; namely, Goss's leaf blight, common corn rust, northern corn leaf blight, and holcus leaf spot in 39%, 82%, 34% and 2% of the fields, respectively. Common corn rust was the most common foliar disease in ND. However, none of the fields had rust levels that would have warranted a fungicide application. Leaf samples exhibiting Goss's leaf blight symptoms were collected and will be used for future research, specifically genetic diversity. Continued survey efforts will help direct corn disease research and Extension efforts, and strengthen disease management recommendations for ND.

Molecular and histochemical analysis of hypersensitive reaction involved in pre- and post-haustorial resistance to *Puccinia triticina*

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Stacking of resistance genes has been suggested as the preferred practice to achieve broader and longer lasting rust resistance in wheat cultivars. However, little is known about the cellular and molecular mechanisms underlying the resistant phenotypes. Moreover, different genes utilize various pathways that result in resistance to a specific race or multiple races. Pre-haustorial resistance generally does not involve hypersensitive response (HR) and is presumed to be more durable and may be involved in broad and non-race specific resistance. Post-haustorial resistance tends to be involved in race specific resistance and is commonly characterized by presence of hypersensitive reaction (HR), which is associated with programmed cell death. In this study, interactions of Thatcher near isogenic lines (NILs) carrying different *Lr* genes with virulent and avirulent races of *Puccinia triticina* were evaluated in time course experiments at seedling-plant stage. Pathogen infection structure development, disease response, and HR, were evaluated in compatible and incompatible interactions. In incompatible interactions, NILs Tc-*Lr9* and -*Lr21* differed in number and time of fungal structure development and H_2O_2 accumulation. Findings from quantification of the expression of HR associated genes in infected plant tissue over time will be presented.

Interactions between *Cadophora gregata* (brown stem rot) and *Fusarium* spp. in soybean plants

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Brown stem rot (BSR) of soybean, caused by *Cadophora gregata* (CG), is widespread in the northcentral U.S. and can cause significant reductions in yield. Soybean plants with BSR symptoms also typically contain other fungal pathogens in the roots. Several *Fusarium* species are especially common, including *F. solani* (FS), *F. oxysporum* (FO), and *F. virguliforme* (FV), the cause of sudden death syndrome. Little is known about interactions between these fungi and how they influence disease development and plant productivity. The objective of this study was to determine how soybean growth and BSR symptoms are influenced by co-inoculations with FV, FO, and FS. Greenhouse trials were conducted with soybean cvs. susceptible and resistant to BSR. The *Fusarium* spp. were mixed into potting soil and seeds were planted. Two weeks after planting, stems were inoculated with types A or B of CG. Foliar disease severity, internal stem browning, and biomass were assessed eight weeks after planting. In all four CG + FV trials, stem browning was significantly increased compared to CG alone. Across the trials of CG +

FV, no significant treatment differences were detected in foliar symptoms. One trial with the BSR resistant variety showed significant differences in biomass between the CG+FV treatment and the noninoculated control treatments. In CR + FO or FS co-inoculations, there was little difference between treatments for the assessments. These studies suggest that interactions can occur between some fungi in soybean and disease severity may be increased by the interactions, but much more work is needed to understand these common interactions and their impact on soybean productivity.

Bacterial and fungal community composition of the wheat phyllosphere and rhizosphere

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Recent research has indicated that the microbes associated with a crop can influence the crop's susceptibility to disease. Several studies have been conducted to investigate the differences in rhizosphere microbiota between crops or land management strategies, but less information is known about the microbiota inhabiting the aboveground tissues of plants. The wheat-associated fungal and bacterial composition was assessed using plants from the Kellogg Biological Station Long-Term Ecological Research wheat/maize/soybean crop rotation site. Barcoded primers were used to differentiate between four land management strategies (conventional till, no-till, reduced nitrogen input, or organic), three growth stages (vegetative, flowering, senesced), and three tissue types (leaf, stem, or root). Collection has been repeated for maize and soybean years. The outcome of this study will describe the tissue-specific communities associated with each plant developmental stage and across all land management strategies. We hope to combine these data with disease incidence data to determine if there is a disease-associated phytobiome.

***Pythium* spp. associated with root rot of celery seedlings in Michigan's commercial greenhouses and fields**

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Michigan ranks second in the U.S. for celery production. In 2014, the farm gate value exceeded \$18 million. Production fields are established with greenhouse-grown seedlings. Root rot of celery transplants caused by *Pythium* spp. is a chronic problem in some greenhouses resulting in poor vigor and an uneven plant stand. Our objective was to identify the *Pythium* spp. associated with root rot so as to improve management strategies. Celery seedlings and maturing plants were scouted from February through July 2014 at five commercial greenhouses and five production fields located in three Michigan counties. Plants of the cultivars Duchess, CR-1 and WA-7 that were stunted with root rot symptoms were returned to the laboratory for pathogen isolation; identification occurred through amplifying the internal transcribed spacers of the ribosomal DNA (rDNA). A total of 46 isolates were obtained (24 and 22 from the greenhouse and field, respectively) and included *P. intermedium*, *P. sulcatum*, *P. mastophorum*, *P. sylvaticum*, *P. oopapillum*, *P. coloratum* and *P. aff. diclinum*. The most prevalent species were *P. intermedium* (33%), *P. mastophorum* (24%), and *P. sulcatum* (17%). *Pythium mastophorum* was isolated from greenhouse seedlings but not detected in the field. Conversely, *P. sylvaticum* was isolated only from field samples. All other *Pythium* spp. were found in both the greenhouse and field. A representative isolate of each *Pythium* spp. was evaluated for virulence using a preemergence (celery seeds) and postemergence (seedlings) bioassay. *Pythium mastophorum*, *P. sylvaticum*, *P. coloratum*, *P. intermedium*, and *P. aff. diclinum* were highly virulent to germinating celery seeds ($P < 0.05$). *Pythium aff. diclinum*, *P. intermedium*, *P. sylvaticum* and *P. mastophorum* were all highly virulent to seedlings ($P < 0.05$) causing root rot and reduced fresh weight; *P. aff. diclinum* was significantly more virulent than all others. Multiple *Pythium* spp. appear to contribute to root rot of greenhouse celery seedlings.

Effect of volunteer corn density on deoxynivalenol production by *Fusarium graminearum*

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Fusarium graminearum causes Gibberella ear rot in corn and produces mycotoxins such as deoxynivalenol (DON). Volunteer corn is a weed resulting from uncollected kernels from the previous harvest, is very difficult to control once established, and can serve as a host for pathogens. An experiment was established in northwestern IN in 2013 and 2014 to examine the impact of volunteer corn density on Gibberella ear rot severity and DON levels in hybrid corn. Two varieties of hybrid corn (one moderately resistant and one susceptible to Gibberella ear rot) were planted. Five different

densities of volunteer corn were established within plots of each hybrid (0, 0.5, 2, 4, and 8 volunteer plants/m²). Experimental plots were inoculated with *F. graminearum* at silking by spreading 500 grams of infested corn kernels in the plot. Hybrid and volunteer ears were collected at harvest, rated for disease severity, and tested for DON. The remaining corn in each plot was combine harvested to collect yield, and a subsample of combined grain was tested for DON. Significantly higher levels of DON were observed in volunteer corn samples compared to hybrid corn samples in both years ($P = 0.0139$). Increasing volunteer corn density did not increase disease severity in either year and disease severity and DON did not differ between hybrids in either year. Yield of both varieties decreased significantly ($P > 0.0001$) with an increase in volunteer corn density. Although disease severity and DON were low in both years, these results indicate that DON could be present and at higher levels in volunteer corn even when DON levels are low in hybrid corn. The trial will be repeated in 2015.

Identification of *Helianthus* accessions resistant to *Plasmopara halstedii* (downy mildew) and *Puccinia helianthi* (rust)

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Downy mildew (*Plasmopara halstedii* (Farl.) Berl and de Toni) and rust (*Puccinia helianthi* Schwein) are two economically important diseases of sunflower (*Helianthus annuus* L.) in the Northern Great Plains. Genetic resistance is an effective tool for managing both diseases. However, resistance genes have been overcome frequently and new sources of resistance are needed. Historically, a disproportionate amount of resistance genes have been identified in germplasm originating from Texas. The objective of this study is to identify new potential sources of resistance to *P. halstedii* and *P. helianthi*. One hundred eighty-two *H. annuus* and 33 *H. argophyllus* accessions originating from Texas were obtained from the USDA North Central Regional Plant Introduction Station and screened to both pathogens in a greenhouse environment. Ninety-three *H. annuus* and 23 *H. argophyllus* accessions were resistant to commonly detected *P. halstedii* races, of which, 18 *H. annuus* and three *H. argophyllus* accessions were resistant to highly virulent races. Seventy-one *H. annuus* and 33 *H. argophyllus* accessions were resistant to commonly detected *P. helianthi* races, of which, 41 *H. annuus* and five *H. argophyllus* accessions were resistant to highly virulent races. Ten accessions resistant to both pathogens were identified and future work will focus on characterizing the genes conferring resistance in these accessions.

Evaluation of chemical treatments and host resistance for management of Sclerotinia stem rot of soybean in Ohio

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Sclerotinia stem rot or white mold of soybean (*Sclerotinia sclerotiorum*) has caused considerable yield losses across Ohio. Since the pathogen is able to survive in the soil for many years and no highly resistant cultivars are available, management of this disease is very challenging. Fungicides and herbicides have shown some reduction in disease severity, however the interaction of fungicides and cultivars with moderate resistance is not fully understood. The objective of the study was to evaluate the effect of fungicides and herbicides applied on soybean cultivars with different levels of resistance to Sclerotinia stem rot. The experiment was conducted in Gustavus Center, OH in 2014. Four cultivars and ten chemical treatments including four fungicides, one herbicide and two application timings (R1 and R2) were arranged in a strip plot randomized block design with eight replications. Disease severity index ranged from 2 to 41 and disease incidence from 3% to 52%. Sclerotinia stem rot developed at some level in all treatment combinations. However, the soybean cultivar with the highest rating for resistance had significantly lowest levels of the disease and the highest yield. Phoenix herbicide and Endura significantly reduced the disease incidence and severity compared to the non-treated control. Based on our initial results, growers should focus on selecting soybean cultivars with the highest resistance rating to manage Sclerotinia stem rot.

Defining the alternative host range and the effect of herbicides on pathogenicity of *Clavibacter michiganensis* subsp. *nebraskensis*, causal agent of Goss's wilt of corn

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Goss's bacterial wilt and leaf blight of corn, caused by the bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), is an annual problem across the Midwest. Several common grass weed species have previously been documented as being alternative hosts of Cmn, and control of these hosts has been presented as a potential disease management strategy. It is important to identify alternative hosts and determine if control of these alternative hosts can reduce inoculum levels. Plants of 14 weed and cover crop species were inoculated in the greenhouse with a suspension of 1.0×10^8 colony-forming units (CFU) of Cmn per mL. Percent of symptomatic leaf area was visually estimated 7 days after inoculation. After 7 days, all plants were subjected to serological testing, examined for bacterial streaming, and leaf tissue was plated onto Cmn-selective medium. Koch's postulates were completed with recovered Cmn isolates, and three new hosts of Cmn were identified: annual ryegrass (*Lolium multiflorum*), johnsongrass (*Sorghum halepense*), and large crabgrass (*Digitaria sanguinalis*). Previously cited hosts shattercane (*Sorghum bicolor*), giant foxtail (*Setaria faberi*), green foxtail (*Setaria viridis*), and yellow foxtail (*Setaria pumila*) were confirmed as hosts. Barnyardgrass (*Echinochloa crus-galli*), cereal rye (*Secale cereale*), and all broadleaf species tested were identified as non-hosts of Cmn. In a separate greenhouse experiment, annual ryegrass, giant foxtail, and large crabgrass were inoculated with Cmn and two weeks after inoculation, plants were treated with seven single mode of action herbicides. Leaf tissue from all plants were examined for bacterial streaming and plated onto Cmn-selective medium. Recovered bacteria were used to inoculate a susceptible corn hybrid to test pathogenicity. Results indicate that herbicide treatment did not reduce pathogenicity of bacteria recovered from infected weed species.

Alternaria spp. from sugarbeet tolerant to four fungicide classes in Michigan

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Alternaria species have caused minor foliar disease issues on sugarbeet historically. Recently, increased incidence and severity of *Alternaria* leaf spot (ALS) has been observed in several growing regions. Once defoliation is evident there is a potential to cause yield loss. One possible factor in the increased disease incidence in sugarbeet fields is that the pathogen has resistance to fungicides used for management of other foliar diseases, such as the major foliar disease Cercospora leaf spot. Fungicide resistance occurs in *Alternaria* spp. on other crops and this could be a factor in ALS on beets. The aim of this work is to test the response of *Alternaria* spp. from beet to commonly used foliar fungicides in the Michigan sugar beet growing region. Eight *Alternaria* isolates from the USDA-SBRU culture collection collected from different areas of the United States between 2002–2009 were included. In 2013 and 2014, *Alternaria* isolates were collected from several sugarbeet fields as part of the annual Michigan Cercospora leaf spot survey. Isolates were single spore transferred and maintained on V8 juice agar. A spiral gradient dilution method was used to estimate the fungicide concentration which caused a 50% inhibition in mycelial growth or spore germination (EC50). All the isolates tested from 2002–2009 showed resistance to azoxystrobin, prothioconazole and thiophanate-methyl fungicides with EC50 >100 ppm. In 2013, five *Alternaria* isolates were tested against different DMI fungicides and Organo tin. Isolates tested also showed resistance to all DMI fungicides (EC50 >100 ppm) as well as moderate resistant to tin fungicide. Isolates from 2014 are still being tested. The finding of high levels of resistance to three widely used classes of fungicides indicates that fungicide resistance is present in the *Alternaria* species on sugar beet and was present even before some of these were registered for use on beets.

Transgenic tobacco expressing a modified spider peptide inhibits the growth of plant pathogens and insect larvae

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The gene encoding lycotoxin I, an amphipathic pore-forming peptide, was modified to increase oral toxicity to insects. One of the most active modified genes was then constitutively expressed in tobacco (*Nicotiana tabacum*) and transformants were evaluated for insect and disease resistance. Pathogenic symptoms caused by *Pseudomonas syringae* pathovar *tabaci* and *Alternaria alternata* on the modified lycotoxin I-expressing leaves were significantly less severe than those seen on wild type leaves. The transgenic leaves also were more resistant to larvae of the corn earworm (*Helicoverpa zea*) and cigarette beetle (*Lasioderma serricorne*) compared to wild type leaves. Gene expression levels of modified lycotoxin I were negatively correlated to the survival of corn earworm larvae. These results indicate that modified

lycotoxin I expression in tobacco can potentially protect leaf tissue from a broad spectrum of pests and pathogens.

Effect of root pathogens *Bioparis sorokiniana* and *Fusarium graminearum* on germination and seedling blight in wheat

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Bioparis sorokiniana (*Bs*) and *Fusarium graminearum* (*Fg*) are important wheat root pathogens that effect seed germination and seedlings establishment and ultimately impact crop productivity. The objective of this study was to explore the effect of *Bs* and *Fg* infested seed on germination and seedlings establishment (blight) of 11 wheat cultivars under greenhouse conditions. Seeds of hard red spring wheat cultivars, Advance, Brick, Briggs, Forefront, Oxen, Prevail, Russ, Select, Traverse, SD4189, and SD4215S, were infested individually with *Bs* and *Fg* by spraying their spore suspension. Infested seed from all 11 cultivars were planted in paper cups (10 seeds/cup) filled with sterile vermiculite, using a complete randomized design. Seed germination and seedling blight data was recorded 10-and 20 days post planting. Wheat cultivars varied in seed germination and seedling blight to both pathogens; however, low seed germination and high seedling blight was observed in *Fg* infested seed as compared to *Bs* infested seed.

Age-related resistance of *Cucurbita* spp. fruit to *Phytophthora capsici*

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Phytophthora fruit rot of winter squash and pumpkin incited by *Phytophthora capsici* is difficult to manage due to prolonged contact of the fruit with soil and the susceptibility of most commercially available cultivars. The fruit of certain *Cucurbita* spp. express age-related resistance (ARR) to *P. capsici* as they develop, however, the onset and degree of resistance is variable. The objectives of this study were to determine differences in the expression of ARR among *Cucurbita* spp. and the effects of exocarp and pericarp firmness on disease incidence. Unwounded, field-grown fruit of 12 hard squash (*Cucurbita moschata* and *C. maxima*) and pumpkin (*C. pepo*) cultivars were harvested at 7, 14, 22, and 56 days-post pollination (dpp) and evaluated for resistance to *P. capsici* after inoculation with a colonized V8-agar plug. All cultivars acquired resistance as they matured, with an absence of lesions on the majority of fruit of *C. pepo* and *C. moschata* cultivars inoculated 22 and 56 dpp. Both *C. maxima* cultivars tested became infected at all maturity levels, displaying characteristic watersoaked lesions with abundant sporangia production four days post-inoculation; sporulation was limited on fruit aged 56 dpp. There was no correlation between exocarp or pericarp firmness and disease incidence among cultivars ($R^2 = -0.01$, $P = 0.85$) aged 22 dpp. Additionally, a puncture or surface wounding of 22 dpp *C. moschata* and *C. pepo* cultivars prior to inoculation consistently resulted in infection, regardless of species. This suggests that resistance is expressed at the exocarp. Germ tubes of *P. capsici* zoospores were observed with light microscopy preferentially infecting via stomata of mature *C. maxima* cultivars. Possible mechanisms for age-related resistance of *Cucurbita* spp. include ontogenetic changes of the cuticle and stomata.

Evaluating resistance of soybean germplasm to *Pythium* species with known resistance to *Phytophthora sojae*

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Pythium species and *Phytophthora sojae* are oomycete pathogens that cause seedling disease, including damping-off and root rot in soybeans. The most effective management tool to reduce stand losses due to seedling diseases is resistance, however relatively little is known regarding resistance in soybean to *Pythium* species. The goal of this project was to screen 47 lines with known resistance to *Phytophthora sojae* for resistance to *Pythium sylvaticum*, *P. lutarium*, *P. irregulare*, *P. oopapillum*, and *P. torulosum*. The cultivars, Archer and Sloan, were used as resistant and susceptible checks, respectively. Two assays were used to screen for resistance: a plate assay, which assesses susceptibility to seed rot, and a cup assay that assesses susceptibility to root rot. Seed rot severity among the lines ranged from 0.75 to 4 where 0 = no seed rot and 4 = seed rotted without germinating. Root rot severity among the parents ranged from 0.87 to 2.91, (0 = no root rot; 3 = severe rot). Some parents showed resistance to seed rot but were susceptible to root rot and vice-versa. A few parent lines showed resistance to multiple *Pythium* spp., but no line was resistant to all five *Pythium* spp. Multiple *Pythium* spp. are often

present in the same field and can cause stand and yield loss. Identification of sources of resistance to multiple *Pythium* spp. that also have resistance to *Phytophthora sojae* would be useful for soybean breeders to incorporate into soybean varieties.

Using RB gene from *Solanum bulbocastanum* to characterize late blight (*Phytophthora infestans*) resistance in potato breeding lines

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Late blight of potato (*Solanum tuberosum* L.), caused by *Phytophthora infestans* (Mont.) de Bary is the most damaging disease of potato throughout the world, because it effects foliage, tuber yield and storability. Previously work demonstrated that the wild diploid potato species *S. bulbocastanum* is highly resistant to all known genotypes of *P. infestans*. In this study, we transformed the RB gene, cloned from *S. bulbocastanum*, into conventionally bred late blight resistant breeding lines, to pyramid late blight resistance genes. All potato transformants were confirmed by antibiotic rooting assays, PCR and RT-PCR for the presence and expression of the RB gene. Foliar host plant resistance was characterized using detached leaf bioassays (DLB), greenhouse whole plant bioassays (WPB) and in field trials. A set of four *P. infestans* genotypes (US-8, US-22, US-23 and US-24) were used to test for resistance in DLB and WPB experiments, and US-22 and US-23 genotypes were used for field trials. Late blight infection was visually measured for percent infection. The results of the DLB and WPB were variable. The greatest resistance was found in some of the conventionally bred, late blight resistant lines, which also contained the RB gene from *S. bulbocastanum*. The field trials showed that some pyramided lines had less foliar late blight than the three non-RB lines used. Genetic engineering of RB late blight resistant lines is a good strategy to study pyramided R-genes in potato.

Metalaxyl sensitivity at two temperatures of *Pythium* spp. from the North Central region

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Several *Pythium* species are known to cause pre- and post-emergence damping off of corn and soybean in the United States. Damping off is predominantly managed using fungicide seed treatments; however, there are reports of reduced sensitivity to active ingredients used in seed treatment products. In addition, temperatures have been shown to affect fungicide sensitivity of *Pythium* spp. The objective of this study was to assess the sensitivity to metalaxyl of *Pythium* spp. from the North Central Region of the United States by assessing mycelial inhibition in vitro. One hundred and twenty isolates of four *Pythium* spp. (*P. torulosum*, *P. oopapillum*, *P. lutarium*, and *P. sylvaticum*), recovered from symptomatic soybean seedlings in the North Central Region, were evaluated for metalaxyl sensitivity across five concentrations (0, 0.1, 1.0, 10, and 100 µg a.i./ml) at 13°C and 23°C using a plate assay. For most isolates, *P. torulosum* was more sensitive (>50% mycelia inhibition) to metalaxyl at 23°C compared to 13°C and *P. sylvaticum* was less sensitive (<50% mycelia inhibition) at 23°C than at 13°C. *Pythium oopapillum* and *P. lutarium* ranged in sensitivity at both temperatures. One isolate each from Arkansas and Indiana was insensitive at 13°C and 23°C across all fungicide concentrations. All isolates of the four *Pythium* spp. from the North Central Region are currently being screened at both temperatures for fungicide sensitivity of ethaboxam, Captan, thiram, and azoxystrobin in vitro.

Characterization of avirulent isolates from the Goss's wilt pathogen *Clavibacter michiganensis* subsp. *nebraskensis*

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Clavibacter michiganensis subsp. *nebraskensis* (Cmn) is the Gram-positive causative agent of Goss's wilt, a bacterial disease of corn. In recent years, Goss's wilt epidemics have exhibited increased disease severity and expanded distribution in North America, designating Goss's wilt a reemerging disease. Goss's wilt has been reported in 18 states and 3 Canadian provinces, and causes grain yield losses up to 50%. Despite the significant agricultural risk posed by Goss's wilt, a mechanistic understanding of Cmn infection remains elusive. Using a Cmn culture library including 175 diverse isolates, the virulence profile of Cmn was determined to be bimodal. While most isolates were highly virulent, many failed to induce disease development. To characterize this disparity, the identities of virulent and avirulent isolates were

confirmed using a combination of genetic and enzymatic analyses including 16S rRNA sequence data and a novel PCR-based assay for specific *Cmn* identification. Putative virulence factors were identified, sequenced, and compared using the reference genome *Cmn* NCPPB 2581. Differential plant-microbe interactions were also explored using inoculations against multiple corn genotypes as well as against the nonhosts *Nicotiana* species and *Arabidopsis thaliana* ecotype KAS-1. In addition, because pathogen metabolism represents an overlooked component of the plant-microbe interaction, 12 representative virulent and avirulent *Cmn* isolates were analyzed with Biolog GP2 MicroPlates and compared to previously reported metabolic profiles for other *C. michiganensis* subspecies. Collectively, the elucidation and identification of pathogen factors contributing to *Cmn* virulence and avirulence will improve our understanding of the genetic determinants for Goss's wilt disease development and facilitate studies of advanced disease diagnosis, pathogen epidemiology, and disease management.

Do *Phomopsis* isolates cause cankers associated with spruce decline?

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Spruce decline is characterized by needle loss and branch dieback, and is most pronounced on Colorado blue spruce (*Picea pungens*). Previous work confirmed that *Phomopsis* is commonly isolated from cankers on spruce exhibiting spruce decline. *Phomopsis* isolates collected from infected trees fell into five groups based on ITS sequence. In order to test if *Phomopsis* causes cankers involved in spruce decline, six spruce species were chosen based on popularity or possible replacements for blue spruce. Norway (*P. abies*), white (*P. glauca*), Black Hills (*P. glauca* var. *densata*), Serbian (*P. omorika*), Meyer (*P. meyeri*), and Colorado blue spruce (*P. pungens*) were inoculated with isolates representing three of the five *Phomopsis* groups isolated from trees exhibiting spruce decline. Trees were inoculated by inserting an agar plug containing *Phomopsis* into a small wound and canker area was measured 78 days post-inoculation. *Phomopsis* isolates from groups 4 and 5 were highly virulent, and similar statistically ($p = 0.31$). Group 3 was less virulent, but still caused small cankers, and controls had no canker expansion. Colorado blue spruce was the most susceptible species while Black Hills and Meyer spruce were resistant but not immune. The remaining three spruce species displayed intermediate levels of susceptibility. *Phomopsis* was successfully re-isolated from all tree species inoculated. Our results confirm that *Phomopsis* can cause cankers on multiple species of spruce and suggests that it may be a major pathogen associated with spruce decline syndrome.

Screening a dry bean Andean diversity panel for potential sources of resistance to *Rhizoctonia* root rot and damping-off

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Rhizoctonia root rot and damping-off, caused by *Rhizoctonia solani*, are among the most economically important root and hypocotyl diseases in the world and affect a wide range of hosts including the common bean (*Phaseolus vulgaris*). To identify potential sources of resistance, screening material was chosen from an Andean Diversity Panel (ADP) based on yield data. Twenty four genotypes were grown in a growth chamber at 22°C and inoculated with an AG4 *R. solani* isolate 14 days after planting. Fourteen days after inoculation, roots were harvested and rated on a scale of 0 (no disease) to 6 (plant dead) and a disease index (DI) calculated. Sixteen phenotypic traits were compared to DI using a correlation matrix and only seed weight was determined to have a significant correlation to DI. These 24 genotypes were mapped onto a neighbor joining tree of 503 ADP lines that had been generated from 4416 SNP markers. Four of the genotypes with the lowest average DI were found to be located within a single clade. Eight genotypes were selected from this clade based on seed weight and screened for disease response as above. Of these eight lines, five (63%) showed reduced disease severity which is an improvement over selection based solely on yield in which only 8 out of the original 24 lines (33%) showed reduced disease severity. Therefore, this correlative approach could provide a promising method for more effectively focusing screening efforts.

Fungicide sensitivity of *Sclerotinia sclerotiorum* isolates from Brazil and USA

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Sclerotinia sclerotiorum is a necrotrophic fungal pathogen that infects more than 400 species of plants. One method of disease control is well-timed fungicide applications. Fungicides such as fluazinam, iprodione, and thiophanate methyl are currently available in Brazil and the USA to manage *S. sclerotiorum* in soybean. To investigate sensitivity of *S. sclerotiorum* to commonly used fungicides, growth of 23 isolates from Brazil (19), USA (3), and Argentina (1) was measured over time at four concentrations of each fungicide amended to PDA. Mycelial growth was measured at 12, 36 and 48 hours post inoculation. EC50, mycelial growth inhibition, and area under mycelial growth (AUMG) were estimated, where the average EC50 was 0.1510 ppm boscalid, 0.0119 ppm fluazinam, 0.2092 ppm fluopyram+prothioconazole, 0.2744 ppm iprodione, and 3.9992 thiophanate methyl. Comparisons using mycelial growth inhibition and AUMG were similar to isolate sensitivity. Interestingly, 10 of 23 isolates failed to produce sclerotia after 40 days growth at the highest concentration of boscalid (15 ppm) and five isolates from Brazil produced little to no sclerotia at 0.5 ppm boscalid. Results showed isolates were sensitive to the fungicides tested in this study, with the exception of one isolate from Brazil with resistance to both carboximide fungicides.

Leaf blight is in the forecast for American ginseng

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American ginseng (*Panax quinquefolius*) is a perennial herb valued for its dried root that is exported to Asia where pesticide residues can create a trade barrier. Ginseng is an important crop in Canada, Wisconsin, and Michigan. Leaf blight, caused by *Alternaria panax*, is an important fungal pathogen of ginseng worldwide, reducing yield and resulting in crop loss. Previous studies with *A. panax* on ginseng indicated that the Tom-Cast forecaster could not be used to effectively limit disease when used in conjunction with chlorothalonil and pyraclostrobin (Hill and Hausbeck 2008). New studies were conducted using the same forecaster but with newly registered and highly effective fungicides. Over two years, four field trials were conducted to compare sprays applied at 7- or 10-day intervals to sprays triggered by cumulative disease severity values (DSVs) of 10 or 15. The trial included two seedling gardens and two two-year-old gardens in 2013 and continued through 2014. In 2013, when disease pressure was relatively low, disease severity was similar for plots treated with the forecaster (10 DSV) and treatments applied every 7 or 10 days. In 2014, when disease pressure was greater, the forecaster (10 DSV) was similar to the 10-day spray schedule, but was not as effective as the 7-day spray schedule. In all cases, the forecaster (15 DSV) resulted in unacceptable disease severity. Yields from two three-year-old gardens were not different from plots treated every 7 days and the forecaster (10 DSV). However, the forecaster (10 DSV) plots had significantly higher yields than plots sprayed every 10 days. Economic analysis of fungicide costs and root yields/value show a significant economic benefit to the forecaster (10 DSV) compared with 10-day sprays. Hill, S.N., and Hausbeck, M.K. 2008. Evaluations of TOM-CAST in timing fungicide sprays for management of *Alternaria* blight on American ginseng. Plant Dis. 92:1611-1615.

Evaluation of *Lysobacter enzymogenes* C3 for control of soybean fungal diseases

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Fungal diseases can cause severe yield losses of soybean. Conventional management approaches utilize fungicide applications, host resistance, and cultural practices to manage fungal diseases, but complete control is rarely achieved. Thus, new alternatives are needed to control fungal diseases of soybean. In this study, we investigated the effectiveness of *Lysobacter enzymogenes* C3, a ubiquitous antifungal bacterium that has shown promise as a biological control agent in previous studies. Results from our research showed that *L. enzymogenes* C3 inhibited vegetative growth or spore germination of several soybean root, stem, and foliar fungal pathogens, suggesting that it has a broad spectrum of inhibiting activities. In laboratory assays, *L. enzymogenes* C3 showed control of root rot diseases caused by *Fusarium virguliforme*, *Phytophthora sojae*, *Pythium sylvaticum*, and *Rhizoctonia solani*. Control of frogeye leaf spot (caused by *Cercospora sojina*) and Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) also was observed. Greenhouse experiments further showed the effectiveness of *L. enzymogenes* C3 in controlling *Rhizoctonia* root rot, sudden death syndrome (caused by *F. virguliforme*), frogeye leaf spot, and Sclerotinia stem rot. Our findings demonstrated that *L. enzymogenes* C3 was effective in controlling many soybean fungal diseases in controlled conditions, and future work will be focused on optimizing its use under field conditions.

Development of a high throughput screening method to examine the fungicide sensitivity of an oomycete soybean community

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Roughly 70% of soybeans planted in the U.S. use a seed treatment as a tool to manage soybean seedling stand establishment issues due to root and stem rot pathogens. Therefore, it is important to examine fungicide sensitivity of *Pythium* spp. and *Phytophthora* spp. on a community scale as numerous species can infect soybean roots, and these species may differentially respond to fungicide seed treatments. However, fungicide sensitivity methods that evaluate radial growth on fungicide-amended agar are slow, labor intensive, expensive and, as a result, are limited to evaluating one or two species at once. Here we present the development of a high throughput microtiter plate assay to evaluate the fungicide sensitivity of an oomycete community to mefenoxam and ethaboxam. Isolates were collected from 12 major soybean-producing states across the Midwest in 2011 and 2012 and consist of 82 different species. Preliminary results indicate that the high throughput assay can evaluate 30 isolates at once, and sensitivities are significantly correlated to those obtained by an amended agar assay. *Pythium* spp. screened varied in response to ethaboxam but were sensitive to mefenoxam. *Phytophthora* spp. screened were more sensitive to ethaboxam than they were to mefenoxam. This research aims to provide data that may influence the chemistries, and their combinations, used for soybean seed treatments.

Characterization and aggressiveness of *Fusarium* species causing soybean root rot in South Dakota

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Multiple *Fusarium* species are associated with root diseases on soybean (*Glycine max* L.) in the United States. In South Dakota the significance of *Fusarium* root rot on soybean has not been recognized. In 2014, a survey of 200 commercial soybean fields covering 22 counties was undertaken in late August through early September in order to realize the following objectives: 1) characterize the frequency of *Fusarium* species causing soybean root rot in South Dakota, and 2) determine the aggressiveness of the *Fusarium* species under greenhouse conditions. A total of 2000 root samples (10 plants per field) were collected between R1 (beginning flowering) and R5 (beginning seed) growth stages. The root pieces were surface sterilized and placed on natural potato dextrose agar (PDA) for 7 days at 25°C under 12-h fluorescent light/dark conditions. Isolates of *Fusarium* species were hyphal-tipped, cultured, and identified based on colony growth and spores produced on natural PDA. Representative *Fusarium* species were confirmed by sequencing the translational elongation factor (EF1- α) gene region and identification via NCBI and *Fusarium*-ID database. Nine *Fusarium* species were identified; among which *Fusarium sporotrichioides* and *Fusarium acuminatum* were the most frequently recovered species. In the aggressiveness study under greenhouse conditions, significant differences in disease severity ($p \leq 0.05$) was observed among the *Fusarium* species with *Fusarium proliferatum* and *Fusarium armeniacum* being the most aggressive (root rot severity >80%). Results from this research will help develop more focused management strategies for *Fusarium* root rot to help soybean producers maximize yield and their return on investment.

Green stem disorder and the effect of fungicide application and stress

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Green stem disorder (GSD) of soybean (*Glycine max*) is when stems remain green and fleshy after pods reach physiological maturity and are ready to harvest. Little is known about GSD and its causes. Although GSD has not been shown to directly impact yield, the immature green stems may lead to harvest issues. The objective of this study was to investigate the effect of stress and fungicide application on GSD incidence. In 2014 in a field located in central Iowa, two soybean varieties had foliar fungicides applied at growth stage R3. Half of the plots had 50% of their pods removed at growth stage R4 to simulate a response to stress. The other half did not have pods removed. Pod removal increased ($P = 0.096$) GSD by over 22%. Fungicides did not affect GSD when pods were not removed. When pods were removed, Priaxor applied at R3 and Aproach applied at R1 and R3 increased GSD by 16 and 19%, respectively. The highest GSD incidence (45%) was observed in soybeans with pods removed and Aproach applied at R1 and then at R3. These results illustrate that fungicide application on soybean plants experiencing stress that causes pod abortion may increase GSD.

Influence of cultural practices on *Diplodia* ear rot, and survival of fungal *Stenocarpella maydis* in infested corn residue

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Diplodia ear rot (DER), caused by the fungus *Stenocarpella maydis*, has become a prevalent corn disease in the Midwest, and the impact of cultural practices on disease management has not been studied in the United States. Soybean-corn and continuous corn rotation systems were evaluated under two different tillage practices under non-inoculated and inoculated conditions at the Agronomic Center for Research and Education (ACRE) in West Lafayette, Indiana. Results from 2014 indicate that cultural practices had a significant effect on yield ($P = 0.0070$). Yields were significantly higher in a soybean-corn rotation compared with continuous corn rotation system under non-inoculated and inoculated conditions. However, crop rotation or tillage practices did not have a significant effect on disease severity under non-inoculated and inoculated conditions ($P = 0.1441$). This experiment will be repeated in 2015. An additional experiment to determine survival of *S. maydis* on corn residue was conducted at the same location. Infested corn residue was either left on the soil surface or buried at 10 or 20 cm. Survival of *S. maydis* was determined by recording presence of pycnidia per unit of corn residue under a stereomicroscope, and assessing spore viability by recording percent germination. Results indicated that after five months, soil depth had a significant effect on *S. maydis* survival ($P = 0.0003$). Pycnidia production and spore germination were only observed on residue left on the surface. Minimal spore germination of *S. maydis* was observed on residue recovered from the 10 cm soil depth (15%), and no pycnidia were recovered from residue buried at 20 cm depth. This research will continue through May 2016.

Assessment of pathogenicity and virulence of *Pythium* spp. in dry bean

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Dry bean (*Phaseolus vulgaris*) is the most globally important food legume. Root rots in dry bean caused by *Rhizoctonia* spp., *Fusarium* spp., and *Pythium* spp. are of significant concern to dry bean producers worldwide. Although several species of *Pythium* are known pathogens on dry bean, some species screened in the study have not yet been tested for pathogenicity and virulence. A cup assay was conducted to determine pathogenicity and differences in virulence. 'Zorro' black bean and 'Red Hawk' kidney bean from the Meso-American and Andean gene pools, respectively, were used to assess pathogen virulence in each of these varieties. Beans were grown in a growth chamber at 20°C and harvested after 12 days for analysis. Three isolates for each of twelve species of *Pythium* were included in the study. To verify pathogenicity in accordance with Koch's postulates, isolations of the pathogen were taken from roots of all inoculated treatments for DNA extraction. Single strand confirmation polymorphism on polyacrylamide gels was used to confirm that the DNA from isolations and the DNA from initial cultures used for inoculation were the same. Some statistically significant yet slight root rot differences existed between the two varieties from the different gene pools, with neither variety consistently showing superior root rot resistance to the other. Four of the twelve *Pythium* spp. tested consistently caused significant reductions in emergence. Four other *Pythium* spp. tested reduced root dry weight by as much as 70 percent. Results indicate that some *Pythium* spp. used in this study that have not previously been reported as pathogens on dry bean may cause root rot in dry bean.

A multiplexed diagnostic assay for detecting *Fusarium virguliforme* and other closely related soybean pathogens

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Soil pathogens belonging to the fungal genus *Fusarium* cause a wide array of crop diseases, including head blight, wilting, brown root rot (BRR), sudden death syndrome (SDS), and many others. Some *Fusarium* species have developed mechanisms to launch themselves onto foliar tissue and infect leaves, stems, and flowers, but most *Fusarium* species infect the root system. Once inside the root system, initial plant symptoms are often quite similar, making it difficult to identify the specific *Fusarium* species causing the disease. Using an isothermal amplification strategy called recombinase polymerase amplification (RPA), we designed primers and probes that can be multiplexed, allowing amplification of unique sequences of the intergenic spacer region (IGS) of *Fusarium* species. This multiplexed design detects seven closely related *Fusarium* species that cause either BRR or SDS throughout the world, and specifically identifies *F. virguliforme*, the causal pathogen of soybean SDS in North America. This assay is an appealing

alternative to other diagnostic tests like quantitative PCR (qPCR) because the reaction time is much shorter compared to the reaction time of qPCR. In addition, this assay is completely portable and can yield reliable results at the point of contact using a crude DNA extraction. The speed and portability of RPA may allow faster management decisions to be made that could potentially minimize yield loss due to BRR and SDS.

RNAi mediated silencing of endogenous wheat genes eIF(iso)4E-2 and eIF4G induces resistance to potyviruses *Wheat streak mosaic virus* and *Triticum mosaic virus*

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Wheat streak mosaic virus (WSMV) and *Triticum mosaic virus* (TriMV) are two viruses affecting wheat in the Great Plains of the United States. The current disease management strategy incorporates the deployment of resistant varieties, mite vector control and various cultural practices; however, it is not fully effective. Both of these viruses belong to the family *Potyviridae* and use host eukaryotic initiation factors in order to facilitate replication of their genomes. We evaluated the use of RNAi to silence eIF(iso)4E-2 and eIF4G to interrupt this process in order induce resistance to these wheat viruses. RNAi expression vectors were independently created from the sequences of the wheat genes eIF(iso)4E-2 and eIF4G. Immature embryos of the wheat cultivar 'Bobwhite' were independently co-transformed by biolistic particle delivery system with RNAi expression vectors and pAHC20, which contains the *bar* gene for glufosinate selection. All progeny have undergone PCR and RT-PCR analysis. To determine viral resistance, the progeny were mechanically inoculated with the viruses. A consistent stable resistance response was demonstrated in three transgenic lines of eIF(iso)4E-2 construct and four transgenic lines of eIF4G, each derived by single seed descent. T6 progeny were co-infected with WSMV and TriMV continue to be resistant. Traditional crosses have been performed with the winter wheat 'Karl 92.' Effectiveness of the RNAi construct has been evaluated using Real-time PCR. Results show up to 18-fold reduction in viral titer in the transgenic lines, the F1 cross and the BC1F1 in compared to control plants. This research provides evidence that a single transgene can provide resistance to multiple viruses and has great potential benefits to both breeders and producers.

Comparison of two screening methods for evaluation of partial resistance to root rot caused by *Fusarium graminearum* in soybean

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Fusarium graminearum is an important cause of root rots of soybean. The most effective method of limiting losses to this pathogen is partial resistance. Screening for partial resistance requires inoculation of seedling roots during germination. Ideally the screening method is simple and economical and produces uniform infection that can be evaluated easily and objectively. Our objectives were to compare screening methods for economy in application and uniformity of symptom expression. Based on previous research we selected the rolled towel assay and the infested rice inoculum method for evaluation. Four soybean cultivars with known susceptibility to infection were inoculated with an aggressive *F. graminearum* isolate. In the rolled towel assay *F. graminearum* conidia in mung bean broth was applied at three concentration rates to surface sterilized seeds. In the rice inoculum assay, infested rice was mixed at five rates, 0, 40, 60, 80, 100:1 in a soil: inoculum mixture. Data collected included root lesion severity, root and shoot length, and root dry weight. The rolled towel assay was simple and economical but results were highly variable and fungal contamination interfered with symptom evaluation. Results of the rice inoculum assay depended on the variable evaluated. Shoot and root lengths were highly variable and poorly correlated with inoculation rates. Root lesion severity and root weights were correlated with inoculation rate and exhibited acceptable variability. Although the rice inoculation method is laborious, it was the most reliable and dependable method of screening for resistance to *F. graminearum*.

Inhibitory effects in-vitro by field adjuvants on *Clavibacter michiganensis* subsp. *nebraskensis*, the causal agent of Goss's wilt and leaf blight of corn

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Inhibition tests were conducted three times in-vitro investigating the potential impacts of spray adjuvants on *Clavibacter michiganensis* subsp. *nebraskensis*,

causal agents of Goss's bacterial wilt and blight of corn. *C. michiganensis* subsp. *nebraskensis* was grown in nutrient broth yeast extract (NBY) broth and aliquots were plated on NBY agar. Filter disks were soaked in one of five adjuvants at one of three concentrations (0.1, 1.0, and 10.0 X label rate) or two controls. Zones of inhibition around the filter disks were measured. Results from chi-square analyses indicated that the magnitude of inhibition varied for all treatments at each concentration on *C. michiganensis* subsp. *nebraskensis* growth ($P = 0.05$). A positive control of copper hydroxide (Kocide® 3000) had the greatest frequency of *C. michiganensis* subsp. *nebraskensis* inhibition among treatments at the three concentrations. Corn producers are more likely to apply adjuvants at label rate (1 X concentration) and results from this study indicate that the non-ionic surfactant (NIS) decreased *C. michiganensis* subsp. *nebraskensis* growth in-vitro compared to the rest of the adjuvants. The adjuvant 10 X concentrations inhibited *C. michiganensis* subsp. *nebraskensis* growth most. NIS inhibited growth more than the copper hydroxide positive control. These results are notable because of the potential for pathogen inhibition by common products that are already in use in corn production. Further research is needed to determine if these effects can be demonstrated in the field and whether differences in chemical composition within an adjuvant group have an effect on *C. michiganensis* subsp. *nebraskensis*.

Impacts of spray adjuvants on severity of Goss's wilt and leaf blight of corn in greenhouse tests

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A greenhouse study was conducted to investigate any potential impacts on disease severity of Goss's wilt and leaf blight, caused by *Clavibacter michiganensis* subsp. *nebraskensis*, when spraying field adjuvants commonly used in corn production. A bacterial suspension of *C. michiganensis* subsp. *nebraskensis* at 1.93×10^8 in 10mM potassium phosphate buffer was spray-infested on Golden Cross Bantam (GCB) sweetcorn plants with an air-pressured canister at V1. One control treatment was applied after plants were infested by wounding them with a floral frog to ensure bacterial virulence. Plants were sprayed with one of five field adjuvants, one negative water control, and one positive copper hydroxide (Kocide® 3000) control 48 hours after infestation. Observations were recorded at 7, 10, 13, 17, and 21 days after spray applications and visual estimates of disease severity were reported. Results indicated greater disease severity when plants were wounded immediately after infestation ($P = 0.05$). There were no differences comparing adjuvant treatments and Kocide® 3000 or the water control for disease severity. Methylated seed oil (Super Spread MSO®) increased disease severity in both trials compared to other adjuvants tested. The Super Spread MSO® had greater disease severity than ammonium sulfate (Bronc®) and high surfactant oil concentrate (High Load®) in the first trial and four treatments including Bronc®, High Load®, crop oil concentrate (ROC®), and a negative water control in the second. These results are notable because of the widespread use of field adjuvants with pesticide applications and the potential for impacts on disease severity in production fields. Further research is needed to determine if these effects occur in the field and if differences in chemical composition within adjuvant groups or varying adjuvant concentrations have similar impacts.

Analysis of soybean promoters' involvement in quantitative resistance to *Phytophthora sojae*

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Phytophthora root and stem rot, caused by *Phytophthora sojae*, is a devastating disease of soybean when production occurs on saturated soils. Host resistance is the most effective means of managing this disease and occurs both qualitatively and quantitatively. Quantitative resistance is not well understood. In order to begin characterizing this resistance, genes associated with quantitative trait loci (QTL) conferring resistance to *P. sojae* were selected for further investigation based on differences in sequence and transcript abundance and/or predicted function. A 2kb region of three promoters was evaluated for sequence differences and for ability to drive GUS expression in resistant and susceptible cultivars following inoculation with *P. sojae*. Sequence information was obtained using Illumina HiSeq™. To investigate GUS expression differences, promoter regions were cloned into binary vectors and inserted into *Agrobacterium rhizogenes* K599 for soybean composite plant production. Transformed roots were stained for GUS expression after inoculation with *P. sojae*. Of the three promoters tested, a putative WKRY and a putative WD40 gene had sequence differences in

resistant versus susceptible cultivars. None of the promoters had a specific pattern of GUS expression. This indicates that the genes these promoters were derived from may not be involved in the expression of quantitative resistance.

Using conditional probability to predict Potato Early Die using *Verticillium dahliae* colony forming units in Michigan commercial potato fields

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A survey of potato (*Solanum tuberosum*) growers in Michigan identified that soilborne pathogens were a concern to potato grower's ability to continue to meet the high demands for potatoes for both the processing and fresh market. Of these soilborne pathogens, *Verticillium dahliae* is one of the most economically important disease from a marketability standpoint due to direct correlation with potato early die (PED), yield decline and its persistence in the soil. A soil evaluation was conducted to study *V. dahliae* interactions with multiple abiotic and biotic factors. The use of geostatistics and geographical information systems (GIS) were incorporated into this study to assess the spatial distribution of colony forming units (CFUs) of *V. dahliae* across fields and to use geostatistical methods to determine *V. dahliae* inoculum levels throughout entire fields. Three fields were used to illustrate this technique. Furthermore, the research team incorporated the use of a nonlinear Kriging method to create conditional probability maps of *V. dahliae* inoculum levels to predict where inoculum levels would result in infection and significant disease incidence and severity. Nonlinear Kriging techniques have advantages over linear Kriging techniques due to their ability to account for uncertainty and are often used to predict the conditional probability for categorical data at non-sampled locations. The methods presented evaluated mapping of soilborne plant pathogens as a potential disease management tool for commercial potato crop production. Conditional probability maps were generated for each field in the study. Field maps spatially represented the probability of PED incidence based on a threshold of 5 CFUs/g soil. The procedures discussed, when incorporated into commercial integrated disease management, will allow for more accurate prediction and treatment of potato diseases, such as PED, associated with *V. dahliae*.

Efficacy of in-furrow fungicides for management of Rhizoctonia root rot of dry bean

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Root rot of dry beans has been a significant issue in North Dakota for several years, causing widespread and substantial yield loss. The absence of above-ground symptoms may not reflect lack of disease, as a plant may appear healthy until removed from the ground. Below ground symptoms can range from red-brown lesions to complete destruction of the root system. Management of root rot traditionally has been through the use of seed treatment fungicides paired with crop rotation, reducing soil compaction, and timely planting, although these practices are not sufficient under high disease pressure. The objective of this research was to evaluate the efficacy of in-furrow fungicide applications for management of dry bean root rot. In-furrow fungicide applications were evaluated in field and greenhouse trials in which dry beans were planted into soil inoculated with *Rhizoctonia solani*. In-furrow fungicides were paired with a standard seed treatment in the field trial. High levels of root rot severity were achieved in both field and greenhouse trials, which accurately depicts disease pressure in many fields. Preliminary field results indicate an overall trend of increased root rot control when in-furrow fungicides were applied, but these differences were not significant compared to the seed treatment alone and no significant yield increase was observed. Preliminary greenhouse results indicated significant reductions in root rot for some in-furrow applied fungicides.

Baseline sensitivity of *Fusarium virguliforme* to SDHI fungicide, fluopyram

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Soybean sudden death syndrome (SDS) caused by *Fusarium* species is among the most devastating soybean diseases in South and North America. In North America, *F. virguliforme* is the predominant causal pathogen of SDS causing approximate 30 million bushels yield loss annually, which ranks this disease within the top-five most yield limiting soybean diseases in the United States. The succinate dehydrogenase inhibitor fungicide, fluopyram, has demonstrated to be successful in management of SDS as a fungicide seed treatment. However, the efficacy of fluopyram to inhibit the growth of *F. virguliforme* mycelia *in vitro* has not been fully explored. In this study, 124 *F. virguliforme* isolates collected from multiple states were included to determine the *in vivo*

mycelia growth inhibition effect of fluopyram. Mycelial plugs were transferred to half strength potato dextrose agar amended with 1, 5, 10, 25, 50, and 100 µg/mL fluopyram in a poison plate assay. Colony areas were measured by scanning the plates at 3 and 10 days after inoculation (DAI), and the relative growth of colony areas between 3 and 10 DAI were used to estimate the relative mycelia growth rate. Effective concentration of half maximal mycelial growth rate (EC50) was estimated in a 4-parameter log-logistic model. Baseline EC50 values for most *F. virguliforme* isolates (n = 119) reside in a range between 1-5 µg/mL with a mean of 2.63 µg/mL, while a small percentage (n = 5) of isolates were less sensitive with an EC50 above 5 µg/mL. Although most of the isolates were sensitive to fluopyram, the minimum inhibitory concentration of fluopyram to *F. virguliforme* could not be detected even a 200 µg/mL concentration was included. Isolate EC50 values were not significantly associated with sampling sites located within or among different states (p = 0.37). These baseline sensitivity values will be valuable in monitoring for fungicide resistance.

Comparative phylogenetic analysis and genetic diversity of *Clavibacter michiganensis* subsp. *nebraskensis*

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Clavibacter michiganensis is a Gram-positive species composed of six plant pathogenic subspecies with different host ranges. Two new subspecies associated with solanaceous plants have been described recently. *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*) is the causative agent of Goss's wilt and blight of corn, the most important bacterial pathogen of corn in the Northern United States and Canada, and can result in up to 50% yield loss. We obtained a diverse collection of *Cmn* isolates from infected fields across the Midwest. Multilocus sequence analysis (MLSA) was used to evaluate genetic variation within a subset containing 50 isolates. Isolates varied by year of isolation (1971–2014), as well as geographic origin and virulence on corn. Four *C. michiganensis* subspecies and a closely related member in the family Microbacteriaceae, *Leifsonia xyli*, were also included in the analyses. Sequencing of three housekeeping genes and one gene known to be associated with virulence in other subspecies of *C. michiganensis* were used to construct a phylogenetic tree using neighbor joining. The grouping of strains was then compared to date of isolation, geographic origin, and phenotypic data. The use of four genes in MLSA was sufficient to differentiate *Cmn* from other *Clavibacter michiganensis* subspecies and revealed *Cmn* to be a monophyletic clade with small but discernible genetic differences among isolates. MLSA provides insights into the genetic variability of *Cmn* and can be used to complement current assays for diagnosis of Goss's wilt.

Susceptibility of onion cultivars to pink root and efficacy of fungicide drenches

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Pink root, caused by *Setophoma terrestris*, is a soil-borne fungal disease that can cause extensive damage on the roots of bulb onions. The objective of this research was to determine the susceptibility of onion cultivars to pink root infection and evaluate fungicide drenches for their ability to limit disease. In 2012, a field study was established in a commercial onion site located in Lansing, Michigan where pink root disease had been a problem in previous years. The commercially available onion cultivars were sown and grown using standard practices. Onions were destructively sampled 110 days after seeding and evaluated for diseased roots (%) using a visual assessment for discoloration. The disease incidence was highest for 'Candy' (36.9%) and 'Highlander' (34.1%) and lowest for 'Redwing' (21.6%) and 'Hendrix' (23.6%). The efficacy of nine fungicides for pink root control when applied as a soil drench was determined in an inoculated greenhouse trial. Six-week-old 'Highlander' seedlings were transplanted into plastic pots containing autoclaved muck soil; millet seeds infested with *S. terrestris* were added to each pot as inoculum. Plant height was measured 49 days post inoculation (dpi), and root density and plant fresh weight were measured 55 dpi. The onion seedlings treated with the fungicide penthiopyrad were significantly larger with a dense root system compared with plants drenched with other treatments and untreated uninoculated plants. However, disease control was not complete because onion plants treated with penthiopyrad were significantly smaller than the untreated uninoculated control plants. The results obtained in this study indicated that yield losses of onion caused by pink root could be limited by selecting less susceptible cultivars. Penthiopyrad may play a role in pink root control but additional studies are needed to determine the optimum timing and application method for the fungicide treatment.