2016 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in St. Paul, Minnesota, June 7–9, 2016. 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: Alam, M., Gleason, M., Zhang, X., and Rehman, A. 2016. Dynamics of stem end rot disease of mango fruit and its management in Pakistan. (Abstr.) Phytopathology 106:S4.184. <http://dx.doi.org/10.1094/PHYTO-106-12-S4.184>

The abstracts are published as submitted. They were not edited at the APS headquarters office.
<http://dx.doi.org/10.1094/PHYTO-106-12-S4.184>
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Dynamics of stem end rot disease of mango fruit and its management in Pakistan

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Stem end rot (SER) is a major threat to the delivery of quality mangoes from Pakistan to markets, particularly through sea-shippments. The dynamics of this disease must be elucidated to devise an effective management plan. The present study sought to understand the infection process and delineate a disease cycle of SER disease under Pakistani growing conditions. Culturing of symptomatic samples (dried branches, twigs, fallen fruits and dried panicles attached to fruit or fallen on the ground) from selected orchards revealed colonization by as many as four pathogens, of which *Lasiodiplodia theobromae* was isolated most frequently. Mature mango fruits were also harvested and stored at 33°C or 12°C for 4 to 5 weeks to facilitate symptom expression. SER was the most prevalent disease on stored fruits, and associated pathogens were identified as *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Phomopsis mangiferae*, *Alternaria alternata* and *Botrytis cinerea*. In subsequent research, the efficacy of fungicides (Amistar®, Sportak®, Scholar®, Tecto®, Nativo®, and Cabriotop®) at different concentrations was evaluated *in vitro* and *in vivo*. Results indicated that Nativo and Cabriotop at 200 and 250 µg/mL provided statistically significant suppression of mycelial growth and SER development.

Reaction of Pakistani elite wheat germplasm to multiple leaf spot diseases

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Leaf spot diseases can contribute significant yield losses to wheat production under favorable environment for disease development. Deployment of leaf spot resistant wheat cultivars seem economical and environment friendly disease management strategy. In this study, 50 wheat genotypes developed by different breeding programs across the country were screened for their reaction to Tan spot (TS), Spot blotch (SB), *Stagnospora nodorum* blotch (SNB) at 2–3 weeks old seedlings in greenhouse. Nine seedlings of each wheat genotype were inoculated individually with *Pyrenophora tritici-repentis* (Ptr) race 1 and race 5 (TS); *Stagnospora nodorum* (SNB) and *Chocliobolus sativus* (SB) spore suspension. Inoculated seedlings were placed in humidity chambers set at 100% humidity for 24 hours for infection initiation. Thereafter, the seedlings were transferred into greenhouse until rated for diseases reaction. Of these 50 wheat genotypes, 28%, 28%, 66%, and 42% were exhibited resistance to tan spot inoculated with race 1, race 5, SNB, and spot, respectively. Interestingly, 3 of the 50 genotypes exhibited resistance to all three diseases and can be used directly or as sources of resistance in the development of leaf spot diseases resistance cultivars to combat TS, SNB, and SB in Pakistan and elsewhere in breeding for leaf spot resistance.

Sub-lethal fungicides induce microsatellite mutation in *Sclerotinia sclerotiorum*

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Stress-induced mutations from sub-lethal fungicide exposure may be an important source of genetic variation for clonal plant pathogens. We used *Sclerotinia sclerotiorum* to assess the role of sub-lethal fungicide exposure in mutation at microsatellite loci. Nine sensitive isolates were exposed to long-term, sub-lethal doses of fungicides with different modes of action: boscalid, iprodione, thiophanate methyl, azoxystrobin and pyraclostrobin. Fungicides were plated in a logarithmic gradient and each isolate sub-cultured from the 50–100% inhibition zone for 12 consecutive generations and experiment repeated. Isolates at the first and twelfth generation from exposed and non-exposed lines were genotyped at six microsatellite loci. Control isolates showed no difference after 12 generations. Five of nine and two of nine fungicide-exposed isolates showed microsatellite mutations in both experiments. One locus did not mutate; all others range from 1 to 34 mutations per locus, with an average of 4.58 stepwise mutations. Most mutations were less than 20 bp, with one 80 bp. The average mutation rate per locus among fungicide-exposed isolates ranged from 4–29 fold greater than the expected mutation rate of 1.3×10^{-5} estimated for microsatellites in *Saccharomyces*. All fungicides resulted in mutations, with iprodione- and azoxystrobin-exposed isolates showing the highest frequency. Results suggest long-term sub-lethal fungicide exposure increase mutation rates.

Improved method for inoculating corn ears

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Corn (*Zea mays* L.) ear rot diseases such as Gibberella ear rot (caused by *Fusarium graminearum*) and Fusarium ear rot (caused by *Fusarium verticillioides*), reduce yield and grain quality annually in the United States. Researching these pathogens and their impact on corn can require inoculation of fungal material into the ears. A new system of mechanically inoculating corn ears was designed using a hydration backpack and auto-filling vaccinator with an attached needle. The efficiency of the new system was compared to a previously established inoculation method using a manual syringe with an 18-gauge blunt end needle attached to a 60 mL syringe, with inoculum carried in a plastic beaker. Inoculation methods were tested by comparing the time to inoculate 100 corn ears with conical suspensions of *Fusarium verticillioides* and *Fusarium graminearum* in a replicated field trial. The new mechanical inoculation system reduced inoculation time by 42% ($P=0.0015$) when compared to the manual syringe and needle method. Additional benefits of the new method include reduced risk of inoculum contamination, consistent inoculum volume per ear, and increased safety for personnel doing the inoculations.

Evaluation of fluopyram as a seed treatment for sudden death syndrome (*Fusarium virguliforme*) management in Nebraska soybean (*Glycine max*) production

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Sudden death syndrome (SDS, *Fusarium virguliforme*) of soybean is becoming more common as a disease in Nebraska soybean production. ILeVO® (ai: fluopyram, Bayer Crop Science) has been shown to have efficacy in managing SDS. In 2015, three on-farm trials were conducted in fields near Fremont, Scribner and Ulysses, NE to evaluate the effect of ILeVO® on SDS development and yield. There were three seed treatments (ST) including non-treated, a base ST, and base ST + ILeVO®. Plots were a minimum of 30 by 1,030 ft with 4 replication of each. SDS incidence and severity were rated at two timings 12 days apart at growth stages R4-R5. Yield was converted to 13% moisture and reported in bushels/acre. Disease incidence was variable throughout the three locations with the highest incidence in Ulysses and Fremont, ranging from 5.7 to 31.1%. The Scribner location had low incidence (5% at the highest) and no treatments were statistically different. At the two locations where SDS was more severe, disease development was significantly lower for the ILeVO® treatment compared to non-treated and there was also a significant increase in yield of 3 - 4 bu/A ($p=0.1$) in the ILeVO® treated plots over non-treated. When SDS is present in a field at higher levels, ILeVO® can be an effective treatment for both managing disease development and reducing yield loss. However, more research is needed to determine an economic threshold level for SDS pressure that warrants the fluopyram seed treatment.

Evaluation of field pea for resistance to, and seed transmission of, Pea Seed-borne Mosaic Virus (PSbMV)

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Pea Seed-borne Mosaic Virus (PSbMV) is a potyvirus capable of reducing yield from 35–82%. In addition to being vectored by aphids, PSbMV also persists in the embryo of the seed and can be transmitted from generation to generation. The rate of seed transmission is directly impacted by PSbMV pathotype, infection timing, the environment and cultivar. The objectives of this research were to evaluate resistance, and determine the rate of PSbMV seed transmission in field pea cultivars commonly grown in North Dakota. PSbMV isolate ND14-1 was obtained from infected field peas grown in North Dakota in 2014. Isolate ND14-1 was mechanically inoculated onto 20 field pea cultivars at 5 weeks maturity. Infection frequency was evaluated at 8 and 11 weeks maturity. In daughter seed harvested at 15 weeks maturity, seed weight reductions ranged from 0–52.4%, which was mainly attributed to reductions in seed size. Based on virus infection frequency, two cultivars were resistant (<15%), one was moderately susceptible (15–40%), and all other varieties were susceptible (>40%) to virus infection. To test PSbMV seed transmission frequency, symptomatic and asymptomatic daughter seeds from infected plants were grown to 5 weeks maturity and tested for the virus. PSbMV seed transmission ranged from 0–60% depending on variety. The presence of seed symptoms did not accurately predict virus transmission.

Epiphytic and endophytic populations and biofilm formation of *Clavibacter michiganensis* subspecies *nebraskensis*, and the relation to virulence

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In 2006, a bacterial disease known as Goss's wilt re-emerged in the Corn Belt of the United States. The causal agent *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*) has an epiphytic phase and it is thought that the bacterium may be able to enter its host through natural openings. To study this, we examined the endophytic and epiphytic survival of two *Cmn* strains that differ in virulence on corn hybrids that vary in susceptibility to Goss's wilt. Inoculated leaf samples were processed directly or first exposed to UVC light to kill surface *Cmn* populations, after which serial dilutions were plated to obtain external and internal populations, respectively. Both populations of the *Cmn* isolates rose over time, however the internal populations of the less virulent strain were significantly lower on the more susceptible hybrid when compared to the more virulent strain. This suggests that the virulence status can affect the ability to develop internal populations. Nineteen strains of *Cmn* were also screened for biofilm formation via two in vitro methods to determine the possible connection of this phenotype to virulence. These strains were previously screened for virulence using two inoculation techniques on Goss's wilt-susceptible corn hybrids. Results indicated variation in biofilm formation among strains. There did not appear to be a correlation of this to virulence of the pathogen, suggesting that biofilm formation may play other roles in *Cmn* ecology.

Pythium and *Phytophthora* spp. isolated from soybean in Minnesota exhibit symbiotic behaviors ranging from parasitism to mutualism

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Oomycetes infecting soybean at low temperatures or in saturated soils are usually associated with root rots, and reduced seed germination, seedling emergence, and final soybean yields. In Minnesota a recent survey isolated 29 *Pythium* spp. and three *Phytophthora* spp. from soybean. Our objective was to compare the effects of known pathogens, *P. ultimum* and *P. irregulare*, apparently beneficial *P. pleroticum* and *P. minus*, and newly isolated *Phytophthora litorale*, *Ph. boreale*, and *Ph. megacarpum*. Inoculation with *P. ultimum* and *P. irregulare* decreased root growth and shoot growth 91 and 86% and 86 and 69% respectively. Inoculation with *P. minus* and *P. pleroticum* increased root growth 8.7 and 3.4% respectively while *P. minus* increased shoot growth >20%. Among *Pythium* spp. and *Phytophthora* spp. isolated in this survey the effect of temperatures on pathogenicity was dependent on species with increasing temperature in some cases increasing pathogenicity. Averaged over three temperatures *Phytophthora* spp. decreased root dry weight 10 to 30% and shoot length 20 to 30%. In soil temperatures where most *Pythium* and *Phytophthora* spp. reduced root and shoot growth selected *Pythium* spp. instead increased growth. Future research should consider the unique characteristics of different *Pythium* and *Phytophthora* spp. The oomycetes *Pythium* and *Phytophthora* spp. exhibit diverse effects on soybean growth ranging from pathogenicity to mutualism.

Effects of fluopyram on soybean cyst nematode 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. Best management practices include using resistant varieties and crop rotation. SCN populations virulent to PI 88788 resistance are becoming more common, warranting additional controls options. The objective of this study was to determine the ability of nematode seed treatments to affect SCN reproduction on different SCN resistance sources. In a greenhouse, 4 seed treatments (Poncho/VOTiVO, fluopyram, Poncho/VOTiVO with fluopyram, and a nontreated control) were compared on 2 resistant varieties, PI 88788 and Peking. A nontreated susceptible (Williams 82) served as a check for a total of 9 treatments. All treatments received a base fungicide seed treatment of penflufen, prothioconazole, and metalaxyl. Using a population virulent to PI 88788 (Type 2.5.7), eggs were serially passed for 3 generations on the same treatment, replicated 6 times, with the experiment repeated (12 total replications). Fluopyram and fluopyram + Poncho/VOTiVO significantly decreased SCN reproduction, females, and eggs/g of dry root, but there was not a significant difference between resistant sources ($p=0.05$). Treatments with fluopyram also had increased vigor and biomass. These results show that seed treatments containing fluopyram may be an effective means of managing SCN resistance when paired with resistant varieties.

Shift in sensitivity of *Alternaria alternata* isolates to famoxadone collected from tomatoes in Michigan

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Alternaria leaf spot is a foliar disease of tomato caused by the pathogen *Alternaria alternata* (Fr.) Keissler and can cause damage to the overall yield and quality of the fruit. Monitoring the fungal population is crucial if fungicide resistance management is to be successfully achieved. Famoxadone belongs to the Quinone outside inhibitor (QoI) (FRAC group 11) class of fungicides that has been used commercially on a variety of crops including tomato. However, mutations occurring in populations of *Alternaria* spp. are reducing the ability of QoI fungicides to effectively control these pathogens. For example, *A. alternata* isolates causing leaf spot of pistachio in California have developed resistance to QoI fungicides due to the G143A mutation in the cytochrome b gene, rendering this fungicide class ineffective. Conidial germination was used to determine the effective fungicide concentration that inhibits spore germination by 50% (EC_{50}). EC_{50} values were determined for a baseline population of *A. alternata* collected between 1999 and 2002 from multiple solanaceous crops throughout the United States, prior to the registration of famoxadone, as well as for isolates collected in 2014 from the Michigan. The mean EC_{50} values for the baseline and 2014 Michigan isolates were 3.1 and 99.8 $\mu\text{g/ml}$, respectively. This indicates that a shift in sensitivity has occurred in the *A. alternata* Michigan population that may extend to other areas of the United States.

Effect of inoculum density of *Rhizoctonia solani* on development of root rot on sugar beet cultivars with varying levels of susceptibility

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Rhizoctonia crown and root rot (RCRR) is a major yield limiting disease of sugar beet in Minnesota and North Dakota. The goal of this study was to determine how inoculum density and host susceptibility influence onset and severity of RCRR disease and yield. *Rhizoctonia solani*-infested barley grain was used as inoculum at 0, 20, 40, or 60 kg/ha. Susceptible (S), moderately susceptible (MS), and resistant (R) sugar beet cultivars were used. Stand counts were recorded weekly beginning at 4 ½ weeks after planting (WAP). There were significant ($P = 0.05$) linear effects for both inoculum density and cultivar susceptibility for all stand counts beginning 4 ½ WAP and significant ($P = 0.05$) inoculum density by cultivar interaction only for the stand count at 15 WAP. There were no significant ($P = 0.05$) inoculum density by cultivar interactions for RCRR rating at harvest, root, or sucrose yields. There were significant linear and quadratic responses to inoculum density for all parameters at harvest. There was a significant effect of cultivar on disease levels at harvest and root and sucrose yields. The R and MS cultivars had lower RCRR ratings, and higher root yield and recoverable sucrose A^{-1} than the S cultivar. The R cultivar had higher percent sucrose and sucrose ton^{-1} compared to the MS and S cultivars. Therefore, selection of a resistant cultivar is very important for planting in fields with a history of *Rhizoctonia*.

Management of soybean root diseases in multiple planting dates and environments of North Dakota

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Field trials were carried out in three diverse environments of North Dakota at Langdon, Minot and Carrington for two years. Planted suitable maturity groups of soybean cultivars after treating with 16 labeled commercial seed treatments as early plantings in mid-May and late plantings in early June and were compared with non-treated control. In Langdon and Carrington the research was done under conventional tillage where as in Minot under no-till conditions. Pre- and post-emergence damping off disease incidence, severity, stand count, yield, and test weight have been recorded and subjected to SAS analysis. The results under multiple environments, different tillage conditions and planting dates showed no significant differences among any of the seed treatments performance in terms of plant stand, disease incidence and severity. However, significant differences in the yield were found among three locations. Likewise, within location between first and second planting. To make a note the yield differences were not due to the disease levels. Mean disease severity range at R2 stage in Carrington was 1.1 to 1.5% in early plantings and 1.4 to 2.8% in late planting date. Whereas, in Minot and Langdon were 0 and 0 to 5.5% in early plantings and 0 to 2.5 and 1 to 6.5% in late planting dates, respectively.

Developing a host-based quantitative PCR assay to detect *Phytophthora sojae* causing root rot of soybean

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Phytophthora root rot caused by *Phytophthora sojae* Kaufmann and Gerdemann, causes severe yield losses in soybean (*Glycine max* (L.) Merr.) globally. In the United States, annual losses exceeded 44 million bushels in 2009. The different phases of *P. sojae* infection elicit the up-regulation and down-regulation of numerous genes involved in pathogenesis. These genes can be used to make accurate and sensitive diagnosis of *Phytophthora* root rot at early infection stage. Phenotypic assay for resistance and susceptibility was done on 23 soybean cultivars (supplied by seed companies) inoculated with a virulent isolate of *P. sojae* (isolate PS-02-30) using inoculum layer method. Based on the phenotypic assay, leaf tissues from six samples showing varied level of *P. sojae* resistance were selected and RNA extracted using the Direct-zol RNA MiniPrep kit. The expression of 11 stress responsive genes in response to *P. sojae* infection was assayed by quantitative PCR to determine their suitability as a diagnostic marker. One gene (Marker Psj-1) out of the 11 selected genes showed an upregulated gene expression in the susceptible cultivars compared to moderately resistant and resistant cultivars. Preliminary results from the gene expression assay suggest this gene may be used as an expression marker for *P. sojae*. Plant pathologists and plant breeders can use this tool for efficient screening for *Phytophthora* root rot resistance in soybean.

Evaluating the efficacy of ethaboxam (V-10208) for control of *Phytophthora capsici* crown and root rot on fresh market peppers

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Fungicides active against *Phytophthora capsici*, which causes crown and root rot in bell peppers, have been effective as soil treatments either applied as a transplant drench or drip irrigation, two methods currently being utilized by growers for fresh market. A new fungicide being developed by Valent U.S.A. Corp. containing the active ingredient ethaboxam (V-10208 4 SC) has shown significant control of a virulent strain of *P. capsici* applied alone or

in rotation with fluopicolide. Ethaboxam was tested in 2014 and 2015 in replicated field trials of bell pepper grown using raised beds, plastic mulch, drip irrigation, and inoculated with *P. capsici*. In 2015, *P. capsici* infestation was severe on untreated plots with a disease incidence of 30% on plants treated with mefenoxam one week post-inoculation. Among ethaboxam treatments, there were no significant differences in severity ratings or incidence for the application types and timings, with greater than 80% control of SAUDPC and only 20% for the mefenoxam treatment. Plots treated with ethaboxam and fluopicolide applied by drip yielded significantly more peppers than a spray treatment using fluopicolide first in the rotation. Ethaboxam, with both fluopicolide and mandipropamid did better than fluopicolide and ethaboxam alone for improving marketable yields.

Differentiation of *Xanthomonas translucens* pathovars in wheat and barley

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Bacterial leaf streak (BLS) of wheat and barley has been of growing concern in areas of small grains production in the Midwestern United States. Of the current 10 recognized pathovars of *X. translucens*, two *X. translucens* pathovars are considered to be of economic importance to wheat and barley growers in Minnesota: *X. translucens* pv. *undulosa* (pathogenic on wheat and barley) and *X. translucens* pv. *translucens* (pathogenic on barley). However, the host ranges of the *X. translucens* group overlap, and many other *X. translucens* pathovars have been demonstrated to be pathogenic on wheat and barley. Currently there are no rapid methods available to determine pathovar designations and it is therefore unclear which *X. translucens* pathovars are present in recent BLS outbreaks in Minnesota. We evaluated multilocus sequencing typing (MLST), multilocus sequence analysis (MLSA) and *in planta* virulence assays for determining the genetic diversity, pathovar designations and host preferences of a large collection of field isolates of *X. translucens* associated with outbreaks of BLS in Midwestern United States. MLSA and MLST corroborated host virulence and was an effective means for assigning *translucens* and *undulosa* pathovar designations.

Occurrence and management of hop (*Humulus lupulus*) powdery mildew (*Podosphaera macularis*) in Michigan

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Powdery mildew (PM), incited by *Podosphaera macularis*, is an emerging threat of hop (*Humulus lupulus* L.) in Michigan. PM was detected in Michigan on 'Yakima Gold' hop in 2015. White, circular, powdery colonies were observed on adaxial leaf surfaces. Microscopic observations revealed chains of barrel-shaped, unicellular conidia (30–35 × 9–11 µm). The 5.8S ribosomal DNA region was amplified with MAT1-1 and MAT1-2 mating type primers (ITS5 and P3). A nucleotide BLAST search revealed 100% identity with *P. macularis* sequences (GenBank accession: AB525917.1). Amplicon sequencing of the 5.8S ribosomal indicated the presence of only one mating type isolate (MAT1-2). Koch's postulates were completed. PM was also observed on 'Newport' and 'Nugget' hop, considered as resistant. Microscopic observations of 'Nugget' hop cones revealed chasmothecia (80 to 82.5 µm diameter) present on bracts, indicating a strong likelihood of two mating types. Fungicidal efficacy was evaluated in the greenhouse on 'Yakima Gold' hop using a randomized complete block design. Eleven foliar treatments were applied at 7- or 14-day intervals. Disease was moderate and control plants developed one or more colonies on 29.6% of their leaves. All treatments limited PM compared to the control. Switch 62.5WDG, Fontelis SC, or Cabrio EG prevented infection but are not currently registered; some treatments resulted in phytotoxicity.

Managing carrot foliar blight caused by *Alternaria dauci* in Michigan using biorationals or TOMCAST

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Michigan growers produce carrots for the fresh and processing markets. In separate studies, we tested biorationals and the TOMCAST disease forecaster in a commercial grower's field as tools to manage foliar blight incited by *Alternaria dauci*. One of four biorationals or copper hydroxide was applied to 'Cupar' carrots every 7–10 days. Plants treated with copper hydroxide had less blight than the control; all biorationals were similar to each other and the control. TOMCAST disease severity values (DSVs) of 15 or 25 were used to trigger fungicide applications and were compared to a 7–10 day spray schedule. Fungicide programs used with TOMCAST included: 1) chlorothalonil alternated (alt.) with azoxystrobin, 2) azoxystrobin alt. with penthiopyrad, or 3) penthiopyrad alt. with cyprodinil+fludioxonil alt. with flupyradifos-methyl+pyraclostrobin. Fourteen sprays were applied using a 7–10 day schedule whereas TOMCAST 15 or 25 DSVs prompted 8 or 4 sprays, respectively. Treatments applied according to TOMCAST 15 or 25 DSVs preserved foliar health and were similar to the 7–10 day schedule; the control showed a moderate level of disease. Fungicide programs tested in conjunction with TOMCAST and the 7–10 day schedule performed similarly and limited disease compared to the control. Results suggest that TOMCAST can be used successfully with a higher threshold (25 DSVs) than reported in earlier studies; 'Cupar' may be less susceptible to foliar blight than previously grown cultivars.

Molecular and cultured-based methods to determine the primary pathogen in the fungal/oomycete complex causing root/crown rot of beans in Mozambique

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Dry edible bean is an important commodity in Mozambique. Recently, root/crown rot (RCR) has caused yield losses in bean fields in Mozambique. A diverse taxa of fungi/oomycetes have been associated to RCR. However, in Mozambique and other countries in Africa there is no information on the causal pathogens associated with RCR. The purpose of this study is to use molecular and culture-based methods to identify the complex of pathogens associated with RCR in Mozambique. A total of 93 RCR samples were collected from two locations (Gurué and Chokwé) in 2014. DNA was extracted from the interface of healthy and infected root/crown area and from FTA cards with imbedded sap from the same plant. DNA was analyzed using Illumina platform, and universal primers (euk7F) were used to amplify the conserved 18S rRNA region. The cultured isolates were observed for morphological features, tested for pathogenicity and analyzed using Sanger sequencing of the ITS region. *Fusarium oxysporum* and *F. solani*, were the most prevalent groups found using the Illumina platform with the most Operational Taxonomic Units (OTU) readings. Culturing potential pathogens resulted in 179 isolates of what 45% were found to be pathogenic. Similarly, the majority of the pathogenic isolates identified through sequencing were within *Fusarium spp.* Relevant pathogenic isolates will be used to screen bean germplasm for RCR resistance.

Genome wide association mapping of resistance to tan spot in durum wheat

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Tan spot, caused by the fungi *Pyrenophora tritici-repentis*, is a major foliar disease on all cultivated wheat crops. The pathosystem involves not only race-specific resistance determined by necrotrophic effector and host sensitivity gene interactions, but also race-nonspecific resistance. Compared to hexaploid wheat, sources of resistance in durum are limited, and genetics of resistance/susceptibility are not well understood. The objectives of this study are to identify sources of resistance and to locate quantitative trait loci (QTL) for tan spot resistance in 429 core durum accessions of USDA-ARS National Small Grain Collection using a genome wide association study. Disease evaluations showed that seventeen lines were highly resistant to all races tested. Sensitivity to Ptr ToxA was mapped to the chromosome 5B as expected, which likely corresponded to the *Tsn1* locus. Using a cutoff value

of $-\log_{10}(p \text{ value}) = 3.0$, we identified genomic regions on the chromosomes 2A, 3B, 4B, 5A, 5B (*tsn1*), 7A and 7B associated with resistance to tan spot. Among them, genomic regions on the chromosomes 3B and 7B were detected for most races indicating race-nonspecific resistance while the others were specific for individual races. The identified resistant lines and the molecular markers linked to resistance QTL will be useful in the development of tan spot resistant durum cultivars.

Comparison of four methods for determination of *Sclerotinia sclerotiorum* fungicide sensitivity

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Several methods are used to determine fungicide sensitivity. The conventional plate-dilution method is time/resource intensive, yet other methods exist: mycelial growth on a gradient, spore germination rate, and germ tube length. To compare, we used 64 *Sclerotinia sclerotiorum* isolates for mycelial growth assays and ascospores from one isolate for spore assays. Five fungicides with four modes of action (MBC, dicarboximide, QoI, and DMI) were used. EC-50 was determined at 5 concentrations (0.001 to 500 ppm) in the plate-dilution method, and using 0.02 to 62.5 ppm in the gradient method. Preliminary results for pyraclostrobin showed a positive correlation between the two methods (slope = 1.341), though not statistically significant ($r^2 = 0.073$). EC-50 determined using ascospores germinated after 5h at 0.001 to 100 ppm and ascospore germ tube length after 20h were compared to mycelial growth inhibition on a gradient. Results showed MBC treatments had no significant difference between methods. Among those that were different, DMI treatments had significantly higher EC-50 for germination ($P \leq 0.040$), dicarboximide treatments had significantly lower EC-50 from germ tube lengths ($P = 0.001$), and QoI treatments had significantly lower EC-50 for both methods ($P \leq 0.030$). Results will be used to determine the best method for large-scale studies characterizing fungicide sensitivity of hundreds of isolates.

Effect of the exopolysaccharide levan on oozing production and emergence by *Erwinia amylovora*

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Erwinia amylovora, the causal agent of fire blight of apple, is disseminated in oozing droplets, which consist of bacterial cells embedded in at least two exopolysaccharides, levan and amylovoran. Levan is a fructose polymer that is synthesized by the enzyme levansucrase, encoded by the *lsc* gene, in the presence of sucrose. Levan production results in prominent domed colonies when grown on medium containing 5% sucrose. We hypothesized that levan production in vivo results in an increase of pressure inside the plant leading to damage of host tissue, ultimately resulting in the emergence of oozing droplets. To study this, an *E. amylovora* *lsc* mutant was generated and was screened for levan production, *lsc* gene expression in shoot tissue and oozing droplets, and was evaluated for oozing production in shoots. This strain was reduced in levan production on both agar plates and in broth containing sucrose and showed a reduction in oozing production during shoot infection. The *lsc* gene was significantly up regulated in 1 cm stem sections at the site of oozing emergence compared to the oozing droplet and the tissue above and below the oozing. For field studies, we screened Michigan strains of *E. amylovora* that were reduced in levan production compared to the virulent strain Ea110. These strains did not show a significant reduction in oozing droplet size, numbers, or population levels in field trials, although many other factors besides levan production could account for these differences.

Exploring the interaction between the bacterial pathogen *Pantoea stewartii* and its maize host

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The ability of *Pantoea stewartii* subsp. *stewartii* (Pnss) to proliferate and cause disease in maize is dependent on WtsE, an AvrE-family type three effector protein translocated from the bacteria via type III secretion into maize cells. In the lab, we examine maize responses to wild-type and *wtsE* mutant strains of Pnss. We previously determined that WtsE causes induction of maize phenylpropanoid gene expression and resulting accumulation of secondary metabolites, including salicylic acid and coumaroyl tyramine. Our most recent studies indicate that WtsE also perturbs plant primary metabolism. In particular, our results suggest that WtsE may play a role in regulating maize glutamate metabolism, as levels of some glutamate-derived compounds differentially accumulate between plants infected with the wild-type versus *wtsE*-mutant strains. To further investigate this, we are currently developing methods for examining the role of individual compounds on bacterial growth and virulence. Here, we show the effects of WtsE on maize primary metabolism, report our progress on extracting maize apoplast contents for analysis of the immediate environment encountered by the bacteria, and detail development of Pnss reporter strains to study bacterial gene expression *in planta* and in response to maize apoplast extracts and specific metabolites. These processes will enable us to define WtsE-dependent maize/bacterial responses as a function of gene expression and metabolism.

Field evaluations of stripe rust (*Puccinia striiformis*) resistance in hard red spring wheat varieties in North Dakota in 2015

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a yield-limiting disease in the wheat (*Triticum aestivum*) growing regions of the United States. Historically, the disease has been most problematic in the Pacific Northwest and Southern regions of the U.S. Since 2000, a new population of *Pst* with shorter latency periods was identified that is adapted to regions that typically have warmer climates. Over the past five years, stripe rust incidence and severity has increased in North Dakota (ND) resulting in yield loss to hard red winter and hard red spring (HRSW) wheat. During the 2015 growing season, a natural infection of *Pst* occurred at five HRSW variety performance trials in ND. Flag leaf area covered by stripe rust pustules was evaluated using a 0–100% scale at each location. A field infection type scale was used at two of the locations. Yield data was obtained from each location and assessments of yield loss were recorded. Flag leaf severity ratings greater than 25% were recorded for 76% of the varieties in at least one location. Based on field infection type scores, varieties that account for over 50% of the ND HRSW acreage were moderately susceptible or susceptible to stripe rust. The lack of genetic resistance in most HRSW varieties indicates a need for additional research on stripe rust management in ND.

Seedling damping-off of sugar beet in Michigan

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Seedling emergence and survival is an important factor in Michigan where as low as 60% stand establishment has been reported in the past. To investigate the cause of seedling losses in the region samples have been taken from fields since 2008. Three pathogens have been determined to predominate in the region: *Rhizoctonia solani*, *Aphanomyces cochlioides*, and *Fusarium* species. Which pathogen or pathogens predominate varies in different years. Other seedling pathogens are found in only a small percentage of beets or in combination with one or more of these three pathogens. Isolates of all of these genera caused damping-off symptoms when inoculated onto sugarbeet germplasm USH20. Differential susceptibility to *Rhizoctonia solani* anastomosis groups has been identified in sugar beet germplasm. All materials screened to date with seedling resistance also have some level of resistance to *Rhizoctonia* crown and root rot as adult plants. Recombinant inbred lines from a cross between a *Rhizoctonia*-resistant and a *Rhizoctonia* susceptible parent showed a wide range of responses, indicating the utility of this material for future studies on the interaction between *R. solani* and sugar beet. Seedling response to *Fusarium* depends on the species used in inoculations.

Screening of wild hop (*Humulus lupulus*) germplasm for identification of resistance to *Pseudoperonospora humuli*

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Pseudoperonospora humuli, the causal organism of hop downy mildew, is considered the most economically-damaging pathogen to hop production. Host resistance, in addition to intensive cultural management practices and fungicide use, is the primary control measure. Wild hops (*Humulus* spp.) are distributed across the North America and Eurasia. They may provide a resource for novel resistance genes and germplasm diversity for local hop breeding programs. Sixteen sites in Minnesota with wild populations of hops were identified. At 14 of these sites individual rhizomes were recovered from 1 – 27 unique individuals. One-hundred six wild and cultivated hop genotypes originating from the USA, England, Japan, and Kazakhstan were screened for foliar resistance to downy mildew (*Pseudoperonospora humuli*) using a detached-leaf assay. Each genotype was tested with three replicates. In total, 33 genotypes were as resistant as the current commercial standard (cv. ‘Teamaker’) which included five cultivars, five Japanese hop (*H. japonicus*) genotypes, two experimental breeding lines, and 21 wild *H. lupulus* genotypes. We identified two geographically distinct locations in Minnesota containing previously unreported germplasm possessing high levels of foliar resistance that may be useful for breeding locally-adapted varieties. Work is currently underway to characterize the genetics, morphological characters and chemical attributes of the hop inflorescence of selected resistant wild plants.

Integrated responses of *Pseudomonas syringae* to light intensity, wavelength, and water availability

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Pseudomonas syringae B728a is a foliar pathogen containing two photosensory proteins that sense blue, red, and far-red light. These proteins regulate swarming motility and contribute to lesion formation and virulence. To understand the light intensities that are biologically relevant, we measured the expression of a light-regulated gene over a range of light intensities. Our results identified increasing responses over the range from 2-20 $\mu\text{mol}/\text{m}^2\text{s}$ blue light, 10-30 $\mu\text{mol}/\text{m}^2\text{s}$ red light, and 2-10 $\mu\text{mol}/\text{m}^2\text{s}$ far-red light. These intensities also influenced the swarming motility of B728a and mutants lacking the photosensory proteins LOV and BphP1. We observed stronger gene expression and swarming responses to far-red light than to red light, indicating that *P. syringae* may be particularly sensitive to far-red light signals, consistent with amplification of far-red light in plant tissues. During its growth on leaves, B728a is likely exposed to light and limited water availability. High levels of drying occur at mid-day when the intensity of blue light peaks. We found evidence that genes encoding a light-mediated signal transduction component and the LOV protein are induced in response to osmotic stress; moreover, this regulation is controlled by AlgU, a stress-responsive sigma factor. The induction of genes encoding photosensory components in response to osmotic stress supports the possibility that *P. syringae* has evolved to respond to co-occurring environmental signals.

Genetic mapping of a gene conditioning resistance to bacterial leaf streak in triticale

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Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (*Xtu*), is an important disease of wheat worldwide. The bacterium can also infect and cause disease on barley, triticale, rye, oat and other grass species. No highly resistant lines in wheat have been reported; however, we have recently identified a number of triticale accessions with an immune reaction or high levels of resistance to the North Dakota *Xtu* strains. In this work, we developed a recombinant inbred line population derived from a cross between highly resistant (L12G09) and highly susceptible (L12G18) triticale accessions. Over 3,000 single nucleotide polymorphism (SNP) markers were obtained using genotyping-by-sequencing (GBS) and mapped to 23 linkage groups. The 23 linkage groups were assigned to specific chromosomes based on the physical locations of the GBS SNP markers, which were obtained by BLAST the tag sequences of GBS SNP markers against the wheat and rye reference genome sequences. QTL mapping revealed a genomic region on the rye chromosome 5R largely associated with resistance to BLS. This is the first report of chromosomal location of a major resistance gene to BLS, and the identified markers tightly linked to the resistance gene will be useful in transferring of the resistance gene into wheat to develop germplasm with high level of resistance to BLS.

Root maceration and sieving improves accuracy of *in planta* nematode quantification

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Quantifying penetration and development of plant-parasitic nematodes (PPNs) in roots is important in understanding interactions between a nematode and its host. A common method to visualize PPNs in roots is to stain with acid fuchsin and observe microscopically. But nematode counts in stained roots can vary greatly due to variability among users, inconsistencies in staining, and variations in root morphology. Also, PPN developmental stages can only be determined based on subjective visual comparisons of the size and shape of stained worms in roots. A newer method to count PPNs is through root maceration and sieving. The efficiency of counting nematodes after maceration was compared to visualization of stained nematodes in roots. Soybean cyst nematode (SCN), *Heterodera glycines*, susceptible soybeans were planted in SCN-infested soil and grown for 15 days. The roots were then stained with acid fuchsin and the number of penetrated SCN were counted microscopically. These same roots were then frozen, thawed, and macerated using a blender. The mixture was poured through a stack of nested sieves and flushed with water. SCN were collected from each sieve and counted. On average, 150% more SCN were counted after maceration than counted microscopically. Also, developmental stages present in the roots were easily isolated and counted, based on body width. Sieves with pore sizes of 250 μm , 150 μm , 75 μm , 45 μm , and 25 μm captured adult females, J4 females, J4 males and J3s, swollen J2s, and parasitic J2s, respectively. Understanding penetration and development of PPNs will allow researchers to learn how root morphology, environmental conditions and nematode-management products such as seed treatments affect plant-nematode interactions.

Evaluation of residual fungicide activity in soybean leaves using analytical chemical quantification and *Sclerotinia sclerotiorum* bioassay

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Sclerotinia sclerotiorum is a pathogen that infects more than 400 species of plants, including soybean. Optimal timing of fungicide applications is dependent on residual activity on/in leaves. Two methods were used to assess residual fungicide activity in soybean leaves: analytical chemical quantification (ACQ) and a detached leaf bioassay (DLB). In order to perform ACQ, ground application was made and about 300 leaves were collected every two days after fungicide application, from day 0 to 14. Extraction were conducted according to salt-induced liquid-liquid extraction followed by LC-MS/MS. For DLB, fluazinam and procymidone were each applied in double application at four irrigation levels and in another experiment, both fungicides and carbendazim were each double applied with/without mineral oil using ground application. Leaves were collected every two days thereafter and inoculated with *S. sclerotiorum*. Results of the ACQ showed the relative concentration of fluazinam decreased from 100% to 7.7% at 14 days after application. Results of DLB for the chemigation experiment showed longevity of fungicide residual varied and higher water irrigation levels more rapidly decreased the residual effect of both fungicides. Use of mineral oil did affect fungicide residual activity after the second application of procymidone. Overall, both methods suggest procymidone and carbendazim has greater longevity and residual activity for white mold control in soybean.

Residual effect of fungicides applied by chemigation for white mold control in dry bean

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Sclerotinia sclerotiorum is a pathogen that causes white mold of dry bean. Chemigation is used for disease control, but effectiveness of this application method and impact of irrigation level on residual fungicide activity in the plant have not been well characterized. Field studies were conducted in three field sites in São Paulo State in Brazil in 2013 and 2014, with applications of fluazinam and procymidone. Fungicide residues were assessed using a detached leaf bioassay, performed by inoculation of leaves with *S. sclerotiorum* and evaluation of lesion area after 48 hours. Area under the disease progress curve estimated from lesion areas showed chemigation at the lowest irrigation level resulted in the lowest total lesion area. Ground applications were no different from chemigation at the lowest irrigation level in most comparisons. Number of sclerotia and dry bean yield were evaluated at Pereiras and showed procymidone application resulted in lower sclerotia than fluazinam in both years, however, yield was only higher for treatments applied with procymidone in Pereiras-2013. Overall, results suggest both lower irrigation level and ground application slow the loss of residual fungicide activity and lessen total lesion area development. There is also preliminary evidence to suggest that procymidone may be better able to reduce *S. sclerotiorum* sclerotia formation, which may be an important consideration for long-term disease management.

The role of phosphodiesterase genes in cyclic-di-GMP degradation and virulence regulation in *Erwinia amylovora*

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Cyclic-di-GMP (c-di-GMP) is a ubiquitous bacterial second messenger molecule, and is an important regulator of virulence in the fire-blight pathogen *Erwinia amylovora*. C-di-GMP strongly up-regulates exopolysaccharide production and biofilm formation, and highly increased levels of c-di-GMP down-regulate motility and virulence. Intracellular levels of c-di-GMP are modulated by diguanylate cyclase (DGC) enzymes that function in the synthesis of c-di-GMP and phosphodiesterase (PDE) enzymes that degrade c-di-GMP. Using a combination of single, double and triple gene deletion mutants, we determined the effect of each of the three predicted PDE-encoding genes in *E. amylovora* (*pdeA*, *pdeB* and *pdeC*), on cellular processes related to virulence. Our findings indicate that all three *pde* genes strongly down-regulate amylovoran production and biofilm formation. In addition, increased levels of c-di-GMP in *E. amylovora* $\Delta pdeC$ coincided with a slower progression of bacterial movement in apple shoots following inoculation in leaves. Moreover, observations from $\Delta pdeA/pdeC$ and $\Delta pdeA/pdeB/pdeC$ deletion mutants indicate the presence of a functional redundancy in the *pde* genes, as motility and virulence were both strongly suppressed in the multiple deletion mutants, but not in the single deletion mutants. These results suggest that intracellular degradation of c-di-GMP is regulated mainly by the genes *pdeA* and *pdeC* in *E. amylovora*.

Effect of rye cover crop on Sudden Death Syndrome of soybean (*Glycine max*), caused by *Fusarium virguliforme*

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Sudden death syndrome is a soilborne disease caused by *Fusarium virguliforme* that negatively affects soybean (*Glycine max*) production in the United States. At present, no management options are completely effective for SDS management. Use of rye (*Secale cereal*) as a cover crop has increased over the past five years in the Midwest, but its impact on SDS is not well understood. A field study was conducted in 2015 to evaluate the effects of rye cover crop on SDS in a long term soybean-corn rotation. Field plots with and without rye as a cover crop were artificially inoculated with *F. virguliforme* before soybean planting, and evaluated for plant stand, root rot, root mass, plant height, disease incidence and severity, and yield. Disease incidence and severity level was low, <15% and <20%, respectively, and no significant differences were observed among treatments on any of the criteria evaluated. In this one-year field study, rye cover crop did not affect SDS, possibly due to low disease pressure. A repetition of this experiment is underway.

Diagnosing for Phomopsis stem canker in sunflower (*Helianthus annuus*) using quantitative polymerase chain reaction assay

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Phomopsis stem canker of sunflower (*Helianthus annuus* L.) is caused by two fungal pathogens in the Northern Great Plains, *Diaporthe helianthi* Muntañola-Cvetkovic, Mihaljcevic and Petrov and *Diaporthe gulyae* Shivas, Thompson and Young. Identification of *Diaporthe* spp. based on morphology on culture media or symptomatic sunflower plants in the fields is challenging. In this study, we developed two TaqMan quantitative polymerase chain reaction (qPCR) assays based on the translation elongation factor 1 alpha (EF1- α) gene region of *D. helianthi* and *D. gulyae* for improved identification of the two *Diaporthe* spp. in sunflower samples. Specificity of the two qPCR assays was demonstrated by using DNA of *D. helianthi*, *D. gulyae*, and other sunflower stem pathogens. The detection limit was determined to be 1 pg of pure *D. helianthi* and *D. gulyae* genomic DNA for their respective qPCR assays. Applicability of the qPCR assays on sunflower field samples was determined by extracting DNA directly from 24 plants collected from commercial sunflower fields in Minnesota, North Dakota, and South Dakota. Additionally, these field-collected sunflower plants were plated onto potato dextrose agar and DNA from the internal transcribed spacer (ITS) region was sequenced from the isolated *Diaporthe* spp. to compare with results from the qPCR assays. Comparative data indicates that the qPCR assays accurately determined *D. helianthi* and *D. gulyae* from infected sunflower tissue.

Inoculation method and watering regime alter symptom expression and relative susceptibility of soybean varieties inoculated with *Fusarium virguliforme*

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Sudden Death Syndrome (SDS) caused by the fungus *Fusarium virguliforme* is an important disease of soybean causing foliar symptoms including interveinal chlorosis and necrosis, and, eventually, complete defoliation. The most effective method of limiting losses is breeding for resistance over multiple quantitative trait loci identified in phenotyping assays that evaluate foliar symptoms of inoculated plants. In earlier research, assays that differed in 1) method of establishing soil moisture and 2) inoculum substrate yielded conflicting results for foliar symptoms. The relative ranking of soybean lines Minsoy and Noir1 was inverted when contrasting watering or inoculation methods were used. Our objective was to determine if differing phenotyping methods can cause expression of spurious symptoms similar to those caused by SDS. This was determined by comparing the foliar symptoms expressed by Noir1 and Minsoy, when resistance assays are conducted using contrasting watering regimes; continuous saturation vs. watering only to field capacity, in differing growth media, and different inoculum substrates, corn meal or sorghum. In flooded silica sand Minsoy expressed the most severe foliar symptoms while in growth media held at field capacity relative foliar symptom severity was inverted. The results indicate that proper controls should be included in phenotyping experiments in order to identify symptoms caused by inoculum substrates and environmental conditions.

Use of a PCR diagnostic system revealed switchgrass rust epidemics caused by two pathogen species differ in temporal dynamics

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Two rust fungi, *Uromyces graminicola* (Ug) and *Puccinia emaculata* (Pe), infect switchgrass (*Panicum virgatum*) in the northcentral region and cause different levels of disease depending on the host population. The epidemiology of disease caused by each species is not well understood due to the difficulty in distinguishing the species during the uredinal stage. A PCR system was developed previously to differentiate the two species utilizing a

primer set designed for Ug coupled with an existing primer set for Pe. Because the Ug primers were found later to amplify DNA from another rust species, a new reverse primer was designed (UgR1: 5'-CTCTTGCTCAACAACAAAATAATC-3') that, when paired with the original forward primer, provides higher specificity to Ug. The PCR system was used to identify the fungus causing early pustules in a Nebraska varietal trial in 2015. From 18 leaf samples containing uredinia collected in mid-July, Ug was detected in 15, whereas Pe was found only in 5. Telia also were observed in 12 samples, and all were identified as those of Ug via teliospore morphology. Microscopic examination of telia in leaves collected in September revealed Pe dominated over Ug in respects to relative numbers of telia. These findings suggest that under Nebraska conditions Ug causes rust in switchgrass and transitions into the resting stage earlier than Pe. They also point to the utility of the PCR system in further epidemiological investigations on switchgrass rusts.

Multiyear evaluation of tactics to manage soybean sudden death syndrome

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Sudden death syndrome (SDS), caused by the soilborne fungus *Fusarium virguliforme*, is a significant disease of soybean. Management of SDS has mainly relied on the use of soybean cultivars with resistance to SDS. However, because infection of roots by *F. virguliforme* soon after seed germination is important for development of high disease severity, seed treatments have been explored as another disease management option. Several fungicidal seed treatments have been developed and registered for SDS management. The goal of this project was to evaluate efficacy of two of those products, either alone or in combination with host resistance, for reducing SDS and increasing seed yield. Studies were conducted with ILeVO® and Heads-Up® seed treatments, alone or in combination with base seed treatments, on soybean varieties with different levels of SDS resistance at irrigated field sites infested with *F. virguliforme*. Studies were in Rosemount, MN in 2013 and 2014, and in Rosemount and Waseca, MN in 2015. SDS incidence and severity combined (Dx score) was significantly lower (up to ~50%) and yield was significantly higher (up to ~13%) with at least one of the seed treatments in each year. The effects of the seed treatments on disease severity and yield were more consistent and greater with the cultivars most susceptible to SDS. In conclusion, these seed treatments, and possibly others, have potential as management tools for SDS.

Integration of resistant cultivars and seed treatment to manage late blight of potato

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Late blight of potato (*Solanum tuberosum*) caused by *Phytophthora infestans* is a significant constraint to potato production. It affects foliage, tuber, yield and storability. Using resistant varieties and treating seeds prior to planting can effectively reduce late blight incidence in seed tubers. Five commercial cultivars and eight advanced breeding lines with foliar resistance to late blight were cut and immersed in a mixture of mycelium and zoospores of the US-23 genotype of *P. infestans* prior to fungicide application. The experiment included two controls (non-inoculated non-treated and inoculated non-treated) and three fungicide treatments (Nubark Mancozeb (NM), Reason or Emesto Silver). Planting was conducted at the Michigan State University Clarksville Research Center, Clarksville, MI. Emergence data was recorded and used to calculate final plant stand (%) and relative area under the emergence progress curve (RAUEPC). Data was analyzed using ANOVA and mean separation by Tukey's HSD ($\alpha=0.05$). The final plant stand and RAUEPC of all treatments and non-inoculated and non-treated control was significantly higher compared to inoculated, non-treated control. Emesto Silver+NM had significantly lower final plant stand and RAUEPC compared to non-inoculated and non-treated control. Reason+NM had significantly lower RAUEPC than non-inoculated and non-treated control, but was not different for final plant stand.

Comparison of succinate-dehydrogenase inhibition, antifungal activity and physical properties of Succinate Dehydrogenase Inhibitor (SDHI) fungicides

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Fungicides are critical for the control of cereal rusts and other diseases. Pathogens such as *Puccinia triticina* are still well controlled by the strobilurins (FRAC Group 2) but major-gene plant resistance is not durable and quantitative resistance genes may be inadequate for some environments and high-intensity agriculture. New products with novel modes of action are valuable for managing resistance development. Several succinate-dehydrogenase inhibitors are on or are entering the cereal fungicide market. Active ingredients such as carboxin, boscalid, penthiopyrad, fluopyram, bixafen, isopyrazam, sedaxane, fluxapyroxad, benzovindiflupyr and penflufen represent several subgroups within this class of fungicides. These active ingredients have a range of intrinsic activity as measured by the respiration inhibition of submitochondrial particles isolated from *Ustilago maydis* with IC₅₀s from 138 nM for boscalid to 0.5 nM for isopyrazam. This study examines the protectant and curative *Puccinia recondita* glasshouse activity and the effects of physical properties on the translation of intrinsic activity to whole plant activity. The correlation of inherent potency to its performance in whole plant activity was impacted by parameters including ACD/LogP and polar surface area. While protectant activity was well-predicted (R^2 adj = 0.93) by intrinsic activity and polar surface area, lipophilicity was a critical additional parameter to predict curative activity (R^2 adj = 0.94).

Characterization of phylogenetic groupings within *Rhizoctonia solani* AG2-2

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Rhizoctonia solani is a heterogeneous group of fungi that have been separated into at least 13 anastomosis groups (AGs). AG2-2 is one of the major pathogenic AG that affects a number of economically important crops including sugar beet (*Beta vulgaris*) and dry beans (*Phaseolus vulgaris*). Traditionally, AG2-2 has been further sub-divided into intraspecific groups (ISGs) that are distinguished by the ability of the culture to grow at 35°C where AG2-2 IIIB grows well at 35°C while AG2-2 IV does not. However, it has recently been shown that these groupings are not phylogenetically supported. In order to clarify these genetic relationships, Martin *et al.* (2014) analyzed the phylogeny of 63 AG2-2 isolates (62 of which were isolated from sugar beet) using four nuclear gene regions and observed three distinct clades. In the current study, we assess 26 of the isolates from this phylogenetic study for virulence on dry beans to test for differences among these newly identified genetic groups. Our data indicates that the average virulence does vary for the three genetic groups with at least one group significantly more virulent than the other two on both adult and seedling dry beans. These results emphasize the importance of proper identification of the genetic group(s) in developing management strategies and testing for plant/pathogen interactions. It also provides the foundation for further work exploring the molecular basis of virulence.

Population dynamics of the biocontrol agent *Lysobacter enzymogenes* in the rhizospheres of soybean and cereal rye

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The soybean cyst nematode (SCN), *Heterodera glycines*, is a major pathogen of soybeans causing significant yield losses. Current management strategies of crop rotation and variety resistance are not completely effective in disease control. Alternative strategies are being explored, where seed treatments with biological control agents (BCA) are available and cover crops, like cereal rye (CR), have been reported to reduce SCN populations. The combination of a cover crop and BCA, however, has not been investigated. This study looked at the potential of the bacterium *Lysobacter enzymogenes* C3 to colonize the rhizosphere of CR and soybean. Seed of CR and soybean were treated with C3 and planted in tubes of soil. Plants were grown in a greenhouse where rhizosphere populations of C3 were determined weekly for 1 month. In repeated experiments, C3 populations on CR, measured on a

per plant basis, increased an average of 1.3 orders of magnitude 2 weeks following planting and then gradually declined. Populations on soybean, however, either showed slight increases or steadily declined after planting. C3 colonized the entire root system of CR, whereas colonization of soybean was restricted to root tips. This suggests treatment of CR seed would be more effective at delivering the BCA into the soil profile than soybean. Since CR is typically planted in high densities, this system could potentially supply sufficient populations of the BCA for an additional level of nematode control.

Building a molecular toolbox to elucidate virulence mechanisms and genomic variability in *Puccinia coronata* f. sp. *avenae*

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Crown rust, caused by the pathogenic fungus *Puccinia coronata* f. sp. *avenae* (*Pca*) is one of the most destructive foliar diseases of oat (*Avena sativa*). The limited resources to manage oat crown rust in combination with the severe yield loss contribute to a steady decline in the acreage planted to oats worldwide. Sexual and clonal populations of *Pca* display high levels of polymorphism, providing an advantage to the pathogen's ability to render deployed resistant oat varieties ineffective. A better understanding of the molecular and genetic factors driving virulence in oat crown rust allows improvements in disease management strategies. To strengthen breeding programs, we have identified a core collection of highly virulent *Pca* isolates to use in resistance screening trials. In parallel, evaluation of oat germplasm and wild relatives with this *Pca* collection is underway to detect novel sources of resistance. In addition, we are investigating the genetic diversity and virulence dynamic of the pathogen using molecular markers and a population of more than 30 *Pca* isolates. Furthermore, we are generating high quality genome assemblies of *Pca* isolates to predict candidate effector genes utilizing machine-learning strategies. Mutagenesis approaches are also in place for functional validation of the predicted effectors. This collaborative research initiative aims to establish the research platforms necessary to uncover the underlying mechanisms of the *Pca* virulence.

Implications of an epidemiological study to management of the soybean cyst nematode in Nebraska

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In Nebraska, corn rotation is a major practice to manage the soybean cyst nematode (SCN; *Heterodera glycines*), the most economically important pathogen of soybean in the Midwest. While it is well established that SCN population densities decline with corn rotation, the factors associated with such a decline in field conditions are not clearly defined. The objective of this study was to identify –using a modeling approach, the major factors affecting SCN population density reduction after annual corn rotation in Nebraska. SCN field population densities (eggs/100 cm³ of soil) were determined before and after rotation in 79 commercial production fields sampled in the state in 2009, 2010 and 2011. After a rigorous field screening and through a careful cycle of model specification, testing and validation (started with a Poisson model), a final model based on the traditional negative binomial was developed. The model showed good performance ($R^2 \geq 0.79$) when validated in a 25, 35 and 50% random sample from the original data set. The model suggested that SCN population levels before rotation and soil pH are major determinants of the population density reduction observed after annual corn rotation in Nebraska. This study is the first to examine relationships of soil factors with SCN during corn rotation and it sets the stage for further studies on effects of pH on SCN.

Exploring the interactions of soybean cyst nematode with stem canker pathogens on soybean

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a serious pest of soybean, *Glycine max* (L.) Merr. In South Dakota, SCN is known to co-occur with the stem canker pathogens, *Diaporthe longicollis* (Hobbs) Santos, Vrandeic and Phillips, and *Diaporthe caulivora* (Athow and Caldwell) Santos, Vrandeic, and Phillips. To explore interactions between *Diaporthe* spp. and SCN on soybean, a completely randomized design using five treatments (*Diaporthe* alone, SCN alone, co-inoculation of SCN and *Diaporthe*, and *Diaporthe* inoculated either 15 days prior to or after SCN introduction) was established in a greenhouse. The soybean cultivar Williams 82 was used for each treatment. Seeds were planted into cone-tainers containing a sand-clay mixture (3:1). Two weeks after emergence, soybean plants were infested with 2,000 eggs/ml of SCN and/or *Diaporthe* mycelial plugs depending on inoculation treatments. At R4 (full pod) growth stage, SCN eggs were extracted from roots and quantified. Stem canker severity was evaluated as a percentage of lesion produced on the soybean stem. We determined that, SCN eggs were reduced by 75% (*D. longicollis*) and 91% (*D. caulivora*) on co-inoculated soybean. In contrast, the SCN eggs were increased by 44% (*D. longicollis*) and decreased by 3% (*D. caulivora*) when SCN inoculation preceded the fungus by 15 days. Information from this research will provide new insights to understanding how multiple pests interact on soybean and how to manage them.

Determining the interaction between the stem canker fungus *Diaporthe longicollis* and the soybean aphid *Aphis glycines* on soybean

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Pests of soybean, *Glycine max* L., are known to co-occur, including the stem canker pathogen, *Diaporthe longicollis* (Hobbs) Santos, Vrandeic and Phillips and the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). We hypothesized that infestations of soybean aphid on the soybean plants can affect the stem canker severity of *D. longicollis* later during the growing season. To test the hypothesis, we took soybean (susceptible cv. Williams 82) and either infested with 5 soybean aphids, inoculated with 4 mm *D. longicollis* agar plug, or both. Soybean were infested at two time points; V1 (inducer) and 6 days afterward (response). At early pod fill (R3) development stage, severity of stem canker was evaluated as a percentage of lesion produced on the stem, and soybean aphid were enumerated. Severity of stem canker was reduced by 39% when infestation of soybean aphid preceded *D. longicollis*, but increased by 29% when the fungus inoculation preceded soybean aphid. There was no significant difference in disease severity between the *D. longicollis* check and the concomitant infestation of *D. longicollis* and aphids ($P \leq 0.05$). Soybean aphid populations were only affected when infestation followed *D. longicollis* inoculation, with populations reduced by 72%. These preliminary results indicate that the soybean defense pathways for these two pests likely differ.

Comparison of visual disease assessment versus GIS/remote sensing methods to accurately detect the epicenters of Soybean rust foci

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A key question in plant pathology forensics is to determine whether or not the introduction of a new plant pathogen was the result of a deliberate, accidental, or natural event. Another important aspect of plant pathology forensics is to locate the epicenters of disease foci to look for forensics evidence, such as the presence or absence of substances that should not be present in the field (e.g. unique isolates, Tween-20, gelatin, syringes, Aluminum foil etc.). Soybean field plots (21 m × 21 m) were established in Quincy, FL. A single soybean plant infected with soybean rust was placed in the center of each plot to initiate disease foci. To quantify the spatial and temporal expansion of soybean rust disease foci, each plot was subdivided into 49 (7 × 7) 9 m² quadrats and each quadrat was visually assessed for disease severity. Focal expansion was also quantified using satellite imagery. Disease severity and satellite image intensity maps were generated and analyzed using ArcGIS 10.3.1 software. Remote sensing/GPS/GIS technologies predicted

the estimated rust foci epicenters to be within 1.5 ± 0.92 m of the actual epicenters. The predicted GPS epicenters, based upon visual severity assessments, were estimated to be 2.46 ± 2.20 m from the actual epicenters. Thus, remote sensing/GPS/GIS technologies have tremendous potential to more accurately and noninvasively determine where (epicenters) to search for forensic evidence within affected fields.

Validation of a warning system for sooty blotch and flyspeck of apple using a modified relative humidity threshold

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Apple growers in the North Central Region spray fungicides on a calendar-based schedule to suppress sooty blotch and flyspeck (SBFS), a fungal disease that blemishes the fruit. A modified warning system based on cumulative hours of relative humidity (RH) above 90 percent was validated over three years (2013–2015) at the Iowa State University Horticulture Research Station using conventional and reduced-risk fungicides. The warning system saved an average of 2.7 fungicide sprays per season compared to the calendar-based control treatment, with equivalent SBFS suppression. Reduced-risk fungicides conferred SBFS control equivalent to that of conventional fungicides. The modified warning system merits wider testing in commercial apple orchards throughout the North Central Region.

Harnessing *Brachypodium* species to identify genetic resistance to oat crown rust

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Yield losses in cereals due to rust infections are a serious agronomic problem. *Puccinia coronata* f. sp. *avenae* (Pca), the causal agent of crown rust, affects oat (*Avena sativa*) production and illustrates the global devastating effect of rust pathogens. Genetic resistance against oat crown rust is limited; therefore the identification of novel mechanisms that may provide durable resistance are pivotal to prevent preharvest crop losses associated with Pca epidemics. Possible strategies to implement durable disease resistance include the introduction of genes from closely related species, or genome editing based on sequence information from other plants. *Brachypodium distachyon* is a small grass species which displays non-host resistance against several wheat rust fungi. Because *B. distachyon* is an experimental model plant for which numerous genetic and genomic resources have been developed, this species may be harnessed to study rust resistance. Here, we propose the amenability of two *Brachypodium* species to identify novel resistance mechanisms against oat crown rust. Using phenotypic and fungal colonization assays and various oat crown rust physiological races we are identifying *Brachypodium* genotypes ideal to dissect the genetic architecture that controls non-host resistance against Pca.

Testing field equipment for the application of *Xanthomonas translucens* pv. *undulosa*, the causal agent of Bacterial Leaf Streak of wheat

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Bacterial leaf streak (BLS) is an emerging disease of wheat in the Upper Great Plains causing significant yield losses. Developing methods to initiate uniform BLS infection is required to screen germplasm for resistance and establish the impact of the disease. Field experiments planted to BLS-susceptible variety Samson were established at Sabin and Crookston in 2014 and 2015 and St. Paul in 2015. Inoculations were applied at the five-leaf stage (Zadoks 15). Three pieces of equipment tested in this study included a CO₂-powered sprayer with a spray boom (4 nozzles), a gas-powered 451 SOLO sprayer fitted with a diffuser screen on a wide angle nozzle, and an 8 jet-spray wheel-mounted boom. In 2015 a 40 jet-spray hand held boom attachment to the SOLO was added. Disease assessments (percent infection on 20 flag leaves/plot) were taken when disease was evident or when plant growth stage was nearing senescence (Zadoks 60–85). Locations within the same year yielded similar results. All sprayer attachments (except the CO₂ boom in 2015) generated significantly higher levels of disease than the non-inoculated and mock-inoculated controls in 2014 and 2015. The CO₂ attachment resulted in the highest BLS severity in 2014, but lowest in 2015, being 16.4% and 4.4%, respectively. Our results suggest that the SOLO sprayer increases disease severity over the CO₂-sprayer in years where conditions for BLS infection are less favorable.

Spatial analyses of fungal-plant interfaces to understand colonization mechanisms of two basidiomycete fungi

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Fungi that colonize plant tissues provide a challenge for morphological and biochemical studies that are focused on the fungal-plant interface. To accurately describe this interface it is necessary to preserve spatial and temporal systems information. This work utilizes several approaches to deepen our understanding of fungal activities that facilitate *in planta* colonization by two basidiomycetes, *Postia placenta* (Pp) and *Puccinia graminis* f. sp. *tritici* (Pgt). Pp is a representative of the brown rot fungi, which circumvent lignin utilization in wood and selectively extract sugars. It is postulated that Pp engages in stepwise plant tissue degradation via staggered enzyme and metabolite release. To examine this hypothesis and validate previously collected transcriptional data, fluorescent *in situ* hybridization techniques are employed to map fungal target transcripts onto colonized plant tissue sections and delimitate explicit fungal reaction zones. The biotrophic rust fungus Pgt releases fungal effectors to orchestrate hijacking of the host physiology and to avoid recognition. Understanding the dynamic interplay between rust and plant host is fundamental to understanding the onset of plant disease. To dissect the fungal-plant interface in this system and to conduct transcriptional profiling of Pgt, laser capture microdissection techniques are under development to ensure collection of infection site enriched material as well as spatial and temporal resolution.

Efficacy of in-furrow fungicides for management of Fusarium root rot in field pea

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Field pea root rot has been a significant concern in North Dakota for several years, causing widespread yield loss. Root rot symptoms include necrotic lesions and reduced lateral root growth, resulting in reduced water and nutrient uptake. Traditional root rot management methods such as crop rotation and timely planting have proven insufficient under high disease pressure. The objective of this research was to evaluate the efficacy of in-furrow fungicide applications for management of Fusarium root rot of field pea. In-furrow fungicide applications were evaluated in field and greenhouse trials where soil was inoculated with *Fusarium avenaceum* or *F. solani*. In-furrow applications were compared to non-treated seed and a commercial seed treatment. High levels of root rot severity were achieved in both field and greenhouse trials, which is representative of disease pressure in many fields. In greenhouse trials, SDHI fungicides fluopyram and penthiopyrad significantly lowered root rot severity and improved emergence under *F. avenaceum* pressure. QoI fungicides azoxystrobin, pyraclostrobin, and DMI fungicide prothioconazole significantly reduced *F. solani* disease severity. Preliminary field results indicated decreased root rot severity when in-furrow fungicides were applied compared to the inoculated/non-treated control, though in many instances, these differences were not significant compared to the seed treatment. No significant yield increase was observed.

Native grasses and common weeds as hosts for *Clavibacter michiganensis* subsp. *nebraskensis*

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Clavibacter michiganensis subsp. *nebraskensis* (CMN) causes Goss's leaf blight and wilt, an important disease of corn in North America. Many alternate hosts have been described for CMN, including grass weeds, a cover crop, and teosintes. Many other plant species, however, have not been tested for their ability to act as hosts of CMN. The objective of this study was to determine if 18 species found in the Midwestern U.S.A. are hosts of CMN. These included widely planted crops and cover crops, weed species common in corn fields, and native grasses. Leaves were inoculated via wounding with CMN in a greenhouse and evaluated for lesion development, bacterial streaming, and CMN population for 3 weeks past inoculation (WPI). Species which developed consistent lesions, showed consistent bacterial streaming from infected tissues, and supported greater than 10^7 cfu/tissue sample of CMN 3 WPI were considered to be hosts of CMN. The grass weed (woolly cupgrass, *Eriochloa villosa*) and the native grasses big bluestem (*Andropogon gerardi*) and little bluestem (*Schizachyrium scoparium*) were newly identified as hosts of CMN. In addition, CMN survived in high populations ($>10^6$ cfu/tissue sample) on non-hosts, including sunflower (*Helianthus annuus*) and common waterhemp (*Amaranthus tuberculatus*) 3 WPI. This study shows that the host range of CMN is larger than previously known and additional plant species may suffer damage and/or sustain populations of CMN in the absence of corn.

Transmission potential of *Sclerotinia sclerotiorum* to cotton seedlings from differential exposure times of seeds to mycelium

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Sclerotinia sclerotiorum causes white mold of cotton (*Gossypium hirsutum* L. var. *latifolium*) and other crops. In production fields, sclerotia and mycelium have been detected within cotton bolls, suggesting that seeds may get exposed to such propagules during continuous or intermittent periods. The association of *S. sclerotiorum* mycelium with cotton seeds is a concern because signs may not be readily apparent and because infected seed is a primary inoculum source. For assessment of seed quality is crucial to understand seed infection and pathogen transmission from seeds to seedlings. This process, though recognized, is not clearly understood. Our study reports the transmission of *S. sclerotiorum* from cotton seeds to seedlings as assessed by seed germination tests and the emergence rate index. Seeds of two cotton cultivars were exposed to fungal mycelium on PDA or PDA plus Manitol Petri plates incubated at 24, 48, 72 and 96 hours. Prolonged seed exposure to the mycelium gradually reduced germination and emergence rate index. Transmission rates from cotton seeds to seedlings were 75.6 and 84.9% in the longest exposure time in both of the tested cultivars. The results suggest that seemingly healthy cotton seeds incidentally or accidentally exposed to mycelium of *S. sclerotiorum* can constitute a risk factor for white mold onset in the field, and potentially, for the development of epidemics.

The following abstract was inadvertently omitted from the 2016 North Central Division meeting abstracts.

Construction and initial characterization of a *dspE* deletion mutant of *Erwinia tracheiphila*

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Erwinia tracheiphila is a xylem-inhabiting bacterium causing wilt of cucurbits in the Northeastern and Midwestern USA. Previous studies have shown that *E. tracheiphila* strains fall into groups exhibiting differences in host-specific virulence, as illustrated by the ability of individual strains to induce rapid wilting on *Cucumis melo* or *Cucurbita pepo* hosts, but not both. An understanding of the genetic basis for such host-specific virulence has been hindered by the lack of effective site-specific mutagenesis systems in *E. tracheiphila*. Here we report the first construction of *E. tracheiphila* mutants. Specifically, we constructed deletion mutants lacking *dspE*, which is an ortholog of genes involved in the virulence of several plant pathogens, and *eopI*, which is an ortholog of a host range-restriction gene in *Erwinia amylovora*. Our mutagenesis methods involved introducing linear PCR products by electroporation into *E. tracheiphila* cells expressing the lambda Red recombinase from the plasmid pKD46. These linear PCR products contained a kanamycin cassette flanked by FRT sites, as amplified from plasmid pKD13, and 50-bp sequences homologous to regions immediately outside the target genes. Inoculation of the *dspE* mutant into *C. melo* plants resulted in significantly reduced wilting, consistent with a role in virulence. Further phenotypic characterization of these mutants is underway, including evaluating the impact of the *eopI* mutation on host-specific virulence.