2012 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in Wooster, Ohio, June 13–15, 2012. 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: Ameen, G., Del Rio, L., and Nelson, B. 2012. Effect of enhanced insensitivity of *Sclerotinia sclerotiorum* isolates to thiophanate methyl on efficacy of disease control. (Abstr.) Phytopathology 102(Suppl. 5):S5.1. <http://dx.doi.org/10.1094/PHYTO-102-9-S5.1>.

Effect of enhanced insensitivity of *Sclerotinia sclerotiorum* isolates to thiophanate methyl on efficacy of disease control

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Phytopathology 102(Suppl. 5):S5.1

A recent study that evaluated the sensitivity of 95 *Sclerotinia sclerotiorum* (Lib.) de Bary isolates to thiophanate methyl (TM), concluded that while most isolates were still very sensitive to this fungicide a few others were less sensitive and some were approaching the threshold that would consider them resistant. To determine whether this enhanced resistance has affected the efficacy of control by this fungicide, a replicated randomized complete block design study was set up in greenhouse conditions. Sunflower plants were sprayed with 0, 1042, 2084, 4168 and 8336 µg/ml concentrations of TM (equivalent to 0, 0.25, 0.5, 1, and 2 times the recommended field doses) and inoculated on the stem with mycelium from six *S. sclerotiorum* isolates that differ in their sensitivity to TM. Lesion length was used to express the reaction of the pathogen to the fungicide. Implications of our findings will be discussed in the poster.

Increase in *Phytophthora sojae* virulence and pathotype number in Minnesota since 1984

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Phytopathology 102(Suppl. 5):S5.1

Phytophthora root and stem rot of soybean caused by *Phytophthora sojae* is an important cause of stand and yield loss in the North Central United States. Resistance based on *Rps* genes is the principal method for controlling these diseases. Reports of *P. sojae* pathotypes capable of overcoming widely deployed resistance genes are becoming commonplace. To assist cultivar development and selection, surveys of *P. sojae* were conducted in 1984, 2000, and 2012. In 1984 only four *P. sojae* pathotypes were identified. In a statewide survey conducted in 2000, *P. sojae* was present in 38% of 91 fields sampled and 12 pathotypes virulent on 4 or more *Rps* genes were isolated in samples from the statewide survey and also from samples submitted to the U of MN Plant Disease Clinic. Of the 23 pathotypes isolated in 2000, 564, 53, 46 and 46% were virulent on *Rps1a*, *Rps1c*, *Rps1k*, and *Rps6* respectively. In 2011 *P. sojae* isolates were collected from soil samples collected at 32 locations where *P. sojae* was considered to be a problem. In this survey the number of pathotypes had almost doubled to 84 pathotypes. Of these 84 pathotypes the proportion virulent on the most widely used resistance genes had increased by 50%. *P. sojae* has proven to be an extremely adaptable organism with the potential for generating highly variable populations in

response to deployment of *Rps* genes. The increasing number of *P. sojae* pathotypes and failure of previously effective resistance genes necessitates integration of management practices and partial resistance to control diseases caused by *P. sojae*.

Use of in-vitro and colorimetric techniques to assay extracts from indigenous plant species for antifungal properties

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Phytopathology 102(Suppl. 5):S5.1

Organically certified agricultural production requires use of natural products as pesticides. Current research to identify natural products that are effective as pesticides relies on expensive and time consuming field experiments. The goals of this research are: 1) To identify compounds obtained from native plants that may have antifungal or antimicrobial properties and 2) to establish the validity of two methods: an in-vitro assay of fungal colony growth rate and a colorimetric absorbance assay sensitive to fungal respiration as reliable indicators of antifungal activity. In the in-vitro assay cultures of the soybean pathogen, *Phytophthora sojae*, were exposed to extracts from Staghorn Sumac (*Rhus typhina*) seed dissolved in nutrient media at concentrations of 100, 200, 400, and 800 ml/L reducing growth of *P. sojae* by 11%, 14%, 22%, and 27% respectively. In-vitro growth of *P. sojae* was negatively correlated with increasing concentrations of extract of *R. typhina*. In the colorimetric assay extract concentrations of 62.5, 125, and 250 ml/L from stems, berries, bark, and new and old leaves were combined in liquid nutrient media containing alamar blue. Light absorbance differed among the extracts from different plant parts indicating differing levels of inhibition and declined with increasing concentration. Absorbance decreased in solutions containing extracts from bark and stems but was unchanged in solutions containing extracts from berries and young leaves. The results indicated that some *R. typhina* extracts inhibit growth and respiration of *P. sojae* and may be a possible source of compounds that inhibit activity of plant pathogens. The in-vitro assay was less variable than the colorimetric assay but required more time and material.

Evaluation of seed treatments for management of *Rhizoctonia damping-off* in carrot

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Damping-off caused by *Rhizoctonia solani* can result significant losses in carrot production. Experiments were performed in a greenhouse to determine the efficacy of seed treatments against this disease. Treatments were Coronet, Coronet+Thiram and Rovral+Thiram. Inoculum of *R. solani* was prepared on chopped potato/soil medium and incorporated into potting mix at the rate of 0.5 g/100 ml mix. The numbers of emerged healthy and diseased seedlings were counted 14, 21 and 28 days after seeding. The experiment was done three times (the first without water misting and the second and third with misting). Emergence was significantly increased compared to the non-treated,

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inoculated control by all seed treatments in all experiments. Pre-emergence damping-off was moderate (33.3, 28.6 and 20.8% in the non-treated, inoculated control in the first, second and third experiments, respectively). All of the treatments significantly reduced pre-emergence damping-off compared to the inoculated control, although there were no significant differences between the effective treatments for the experiments without misting. The low rate of Coronet alone significantly reduced pre-emergence damping-off compared to the other treatments for the experiment with misting. Post-emergence damping-off was relatively low (2.6, 5.2 and 9.9% in the non-treated, inoculated control in the first, second and third experiments, respectively). All of the treatments significantly reduced post-emergence damping-off and increased fresh weight of surviving carrot seedlings compared to the inoculated, non-treated control.

Comparison of methods and corn root types for efficient extraction of endoparasitic nematodes

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Endoparasitic nematodes, such as *Pratylenchus* spp. (root-lesion) and *Hoplolaimus* spp. (lance), annually reduce the yield of corn in the United States. Previous research showed that nematode population densities were highly variable and extraction methods may not provide consistent results. In order to determine the most consistent and time-efficient method, comparisons were made between four nematode extraction techniques, corn root types, and extraction times. The extraction methods evaluated were aeration-incubation (AI), a modified Baermann funnel (BF), Seinhorst's mister (MI), and shaker incubation (SI). Research plots were established in two Nebraska corn fields in 2009 and 2010 with histories of high population densities of these endoparasitic nematode genera. Nematodes were extracted from six root types with the four extraction methods for 3, 5, and 7 days in a complete factorial treatment design. In 2009, the highest population densities of *Pratylenchus* spp. were extracted from the first set of anchor roots with either the BF or in the AI ($p < 0.0001$). *Hoplolaimus* spp. had the greatest recovery rate from seminal roots on the Baermann funnel in 2009 ($p < 0.0001$). The MI assembly malfunctioned in 2009 and results were omitted from analyses. In 2010, fine feeder roots in MI or AI had the greatest recovery of both genera of endoparasitic nematodes ($p < 0.0001$). After 7 days incubation, it was shown that 50% or more of the total nematode population densities could be extracted with as few as 3 or 5 days incubation. For most root types, extraction timings, and nematode genera, the SI method extracted the fewest nematodes per gram dried root ($p < 0.0001$). Based on these results, it can be concluded that nematode extraction efficiency varies by genus for root type and extraction method.

Characterization of fungicide resistance in *Cercospora beticola*

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Cercospora leaf spot (CLS) of sugar beet is an important foliar disease worldwide. Disease is mainly controlled by fungicides like demethylation inhibitors (DMIs) and quinone-oxidase inhibitors (QoIs). The purpose of the study was to identify the molecular basis for DMI and QoI resistance. DMI act by inhibiting cytochrome P450-dependent sterol 14 α -demethylase (CYP51), which is required for biosynthesis of the cell membrane component ergosterol in fungi. The study was initiated by cloning CbCyp51 gene and carrying out sequence analysis from isolates with varying EC50 values. However, no mutations were associated with DMI resistance. No evidence of alternative splicing of CbCyp51 was found that might explain reduced sensitivity to DMIs. However, CbCyp51 was overexpressed in isolates with high EC50 values than the isolates with low EC50 values. After exposure to tetraconazole, isolates with high EC50 values responded with further induction of CbCyp51, with a positive correlation of CbCyp51 expression and tetraconazole concentration up to 2.5 μ g ml⁻¹. QoIs, on the other hand target cytochrome b gene which encodes for a protein that forms the core of the mitochondrial bc1 complex. The Cbcyb gene was cloned and sequence analysis of Cbcyb was carried out in QoI-sensitive (<0.080 μ g ml⁻¹) and QoI-resistant (>1.0 μ g ml⁻¹) isolates. All tested QoI-resistant isolates harbored a point mutation in Cbcyb at nucleotide position 428 that conferred an exchange from glycine to alanine at amino acid position 143 (G143A). A PCR assay was also developed to discriminate QoI-sensitive and QoI-resistant isolates based on the G143A mutation. Our results indicate that QoI resistance has developed in some *C. beticola* populations and monitoring the G143A mutation is essential for fungicide resistance management in this pathosystem.

Occurrence and distribution of *Triticum mosaic virus* in the central Great Plains

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Phytopathology 102(Suppl. 5):S5.2

Wheat curl mite-transmitted viruses namely *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and *High Plains virus* (HPV) are the most common viruses infecting winter wheat in the central Great Plains of the United States. Field surveys of winter wheat were carried out in Colorado, Kansas, Nebraska, and South Dakota in the spring and fall of 2010 and 2011 to determine the prevalence and incidence of WSMV, TriMV, and HPV. In each state, up to 31 counties per state, three fields per county, and 50 tillers per field were sampled and tested for the presence of TriMV, WSMV, or HPV using ELISA. WSMV was the most prevalent and was detected in 83% of 185 season-counties (= counties), 74% of 420 season-fields (= fields), and 36% of 12,973 samples. TriMV was detected in 32, 6, and 6% of counties, fields, and samples, respectively. HPV was detected in 34, 15, and 4% of counties, fields, and samples, respectively. TriMV was detected in all four states. Ninety one percent of TriMV-positive samples were co-infected with WSMV whereas WSMV and HPV were mainly detected as single infections. Incidence of each virus occurring in a triple infection was <5%. The high frequency of TriMV and WSMV double infections has implications on the epidemiology and management of the two viruses.

Effects of microbial inoculants on plant growth promotion

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The expansion of organic agricultures has increased demand for alternatives to chemical fertilizers and pesticides. Here, we report the results of two series of greenhouse bioassays, testing plant growth promotion stimulated by experimental and commercially available products. Specifically, we assessed the effects of drench treatments of *Burkholderia* sp. Strain R4F2, *Mitsuraria* sp. Strain H24L5A, in comparison with a commercially-available *Streptomyces* inoculant (Actinovate). Additionally, we assessed the effects of an algae-based biofertilizer (ABB2) in comparison to the commercial algae-based biofertilizer product Restart. Moreover, we also tested the commercial products Abundance and AZO. Treatment effects on root and shoot biomass and root and shoot length were assessed on seedling growth. Of the bacterial treatments, *Mitsuraria* sp. (10⁷ cells/ml) and *Burkholderia* sp. (10⁷ cells/ml) provided the greatest enhancements of shoot growth as compared to the negative controls ($P < 0.05$) in Tomato Celebrity F1. In contrast, no significant responses to the bacteria applied to Organic RB07 Hard Red Spring wheat were observed. Of the algae treatments, both experimental and commercial treatments were comparable (ABB2 (1X) and ReStart (1X)), with shoot length increasing in tomato ($P < 0.05$), but not wheat. These data indicate the plant growth promoting activities of the experimental ingredients, and indicate that these unformulated inoculants have utility comparable to that of commercial formulations. Additional work on refining formulations is expected to further enhance efficacy and provide evidence of the commercial potential of these experimental microbial inoculants as well as biocontrol activity.

Assessing the efficacy of the fluorimetric probes dichlorofluorescein (DCFH) and Amplex Red in estimating reactive oxygen species (ROS) in ash (*Fraxinus* spp.)

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Reactive oxygen species (ROS) are by-products of photosynthesis and respiration in plant tissues. Attack by herbivores and pathogens, and abiotic stress, also induce the production and transient accumulation of ROS in plants, whereby they can act as secondary messengers/chemical mediators in plant defense signaling and lead to programmed cell death. The emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB, Coleoptera, Buprestidae) is an invasive insect pest that has been killing millions of susceptible North American ash trees since its discovery in Michigan in 2002. However, Manchurian ash (*Fraxinus mandschurica* Rupr.), a native species from China, is resistant to EAB. We hypothesize that ROS are induced by EAB attack in Manchurian ash at higher levels than in susceptible ash species. Testing this hypothesis

requires a method to quantify low-level ROS that is not available at present. In this study, we explored the efficacy of the fluorimetric probes dichloro-fluorescein (DCFH) and Amplex Red for measuring ROS in Manchurian and black ash (*Fraxinus nigra* Marshall) – a susceptible species. Trees were placed in a randomized complete block design. Half of the trees from each species were water stressed for seven weeks. EAB eggs were applied on the main stem of half of the trees in each water treatment. Phloem tissue was collected 14 days after egg application and extracted in phosphate buffer. We achieved nmol/g FW quantification of ROS and observed wider linear dynamic range for Amplex Red than for DCFH. The Amplex Red assay is still being optimized, but with DCFH we found significantly higher levels of ROS, away from the induction site, in Manchurian than in black ash. Treatment had no effect, but the interaction between species and treatment was significant. We are currently analyzing ROS in the tissues collected from the insect feeding zone.

Calcium signaling pathway plays an important role in fungal virulence in plants and humans

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Phytopathology 102(Suppl. 5):S5.3

The Ca²⁺ dependent signaling pathway plays an important role in cellular development and metabolism. Previous studies have shown that Ca²⁺/calcineurin signaling is essential for pathogenicity in *Magnaporthe oryzae*, a causing agent of rice blast. MoCRZ1 (*M. oryzae* Calcineurin Responsive Zinc Finger) acts as a downstream regulator in Ca²⁺-dependent signaling for gene expression regulating calcium homeostasis and pathogenicity in this fungus. MoCRZ1 null mutant showed reduced virulence and less tolerance to calcium stress. Research was done to identify genes that are regulated by MoCRZ1 by ChIP-chip and verified by microarray. Interestingly, in *Aspergillus fumigatus*, a primary and opportunistic pathogen as well as a major allergen that is readily isolated from human habitats, CRZA (*A. fumigatus* Crz1 homolog) acts in a similar pattern as MoCRZ1 in this fungus. The crzA mutant showed less cellular tolerance to heat and ion, loss of virulence when compared with wild type strains and also altered expression of calcium transporter mRNAs under high concentrations of calcium. To identify genes regulated by CrzA, ChIP-seq was done to identify binding sites. 102 genes were identified and compared with *M. oryzae* MoCRZ1 regulated gene list for the purpose to find a common regulatory theme between these two different fungi.

Engineering an attenuated strain of Pepino mosaic virus effective for cross protection

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Plant viruses cause serious economic losses both in many major crops. Current management approaches, including breeding for virus-resistant varieties, controlling virus-transmitting insects, and planting virus-free seed stocks, have met with limited success. Cross protection, the practice of mitigating virus-caused crop losses by pre-inoculating seedlings with an attenuated strain (a plant virus “vaccine”) of the same virus represents a viable alternative. So far, all known cross protection-inducing vaccines are field isolates associated with milder symptoms which could evolve to become more virulent. Therefore, the absence of a rapid and reliable procedure for developing attenuated plant virus vaccines represents a major obstacle for widespread use of cross protection. The aim of our study is to develop a knowledge-based approach to produce predictable and stable vaccine strains. We used Pepino mosaic virus (PepMV), an emerging pathogen of tomato, as the model virus for our investigations, and chose its capsid protein (CP) gene for modification to produce an attenuated strain. We first compared the PepMV CP with the CPs of other closely related viruses to identify the less conserved amino acid (AA) residues within the CP gene. These less conserved AA residues were then replaced with their counterparts in a different virus (Potato virus X) and the derived PepMV mutants were tested for their ability to cause diseases in *Nicotiana benthamiana* plants. One of the mutants, referred to as KDVC, was found to retain the ability to infect the plants systemically yet cause very mild symptoms. This mutant was further found to protect the plants against secondary infection of the wild-type PepMV. Our results established a reliable procedure for producing attenuated viruses for use in cross protection. We expect this approach to be applicable to other plant viruses and lead to improved productivity in major crops prone to virus diseases.

Metabolite profiling to predict coast live oak resistance to *Phytophthora ramorum*

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Over the last decade, high mortality rates have been reported in California in natural populations of coast live oak (CLO; *Quercus agrifolia*) as a result of *Phytophthora ramorum*. In spite of high mortality rates, asymptomatic CLO have persisted. Though the mechanisms of persistence are not known, putative phenolic biomarkers of oak resistance to the pathogen have been identified and threshold levels quantified. To test the accuracy of these biomarkers in predicting resistance within natural populations of CLO, we haphazardly selected 150 trees from a naïve stand in the East Bay Regional Park District, Contra Costa Co., California, and sampled them for constitutive biomarker analysis. Trees were then inoculated with *P. ramorum* to assess their resistance, by measuring resulting cankers approximately ten months after inoculation, and to relate it to the putative biomarkers. Based on a previously developed statistical model incorporating canker length, 14% of trees have an 80% probability of survival, i.e. of being resistant; however, not every resistant tree contained a phenolic biomarker at or above threshold levels. Therefore, additional work must be done to identify individual or groups of biomarkers that can predict resistant trees more accurately. Our results will help inform efforts aimed at mitigating the disease via exploitation of host resistance, which is one of the most effective and economically feasible disease management strategies for trees in forested ecosystems.

Wheat blast: An emerging threat

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Wheat blast, caused by the *Triticum* pathotype (Mot) of *Magnaporthe oryzae*, was first identified in Brazil in 1985, and blast has since become a serious constraint to wheat production in Brazil, Bolivia and Paraguay. Although South American (S.A.) Mot strains have not been identified in the U.S., a closely-related, native *M. oryzae* turf grass pathogen (*Lolium* pathotype, Mol) was isolated from a single blasted wheat head in Kentucky in 2011. Thus wheat blast could become established in the U.S. through a host shift from native Mol strains or through introduction of S.A. Mot strains. To date, we have screened 418 US winter wheat varieties and 80 spring wheat varieties for blast resistance in Biosafety Level-3 containment facilities at Ft. Detrick and KSU. Using historical and freshly-collected Mot strains, we have observed a continuum in head blast susceptibility among U.S. varieties, with some showing moderate to high levels of resistance. The first field test of U.S. varieties with natural blast populations and environmental conditions has been performed in Quirusillas, Bolivia. No evidence has been found for the presence of physiological races among isolates used so far. Diagnostic primer sets are being developed based on genome sequences from Mot, Mol and other host-specific *M. oryzae* populations. A quantitative pathway analysis is underway to estimate the probability of Mot entry and establishment in the U.S. through importation of wheat grain from two blast endemic areas in S.A.: Parana and Rio Grande do Sul, major wheat producing states in Brazil. This analysis may help inform regulatory policy for U.S. wheat imports from at-risk countries, and help develop preparedness plans for early detection, accurate diagnosis, and effective mitigation. A wheat blast website (<http://www.ksu.edu/wheatblast>) has been launched as a resource for the wheat community.

Axoxystrobin resistance in *Alternaria* spp. from potato

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Potato early blight caused by the fungus *Alternaria solani* is a perennial and potentially destructive disease in WI. The causal agent of potato black spot, *A. alternata*, can also be found in WI potato fields, yet its role and implications are, to date, unclear. Primary symptoms of both diseases in WI include dark brown to black and circular lesions on leaves with distinctive target patterning within lesions. Lower leaves are first infected and, left unmanaged on susceptible varieties, can lead to premature defoliation with yield and quality losses. There are effective fungicides for control of both potato diseases. However, the strobilurin or QoI (quinone outside inhibitor) class of fungicides have a single-site mode of action with high risk for resistance. QoI resistance

develops through mutations in the cytochrome b gene at 3 possible sites. The mutation of an isolate affects the type of resistance it will express. Isolates with mutation at site G143A express complete resistance; mutations at sites F129L or G137R express partial resistance. To prolong efficacy, high resistance risk fungicides are used preventively in alternation and tank-mixes at appropriate rates. Despite management efforts, resistance of *A. solani* and *A. alternata* to azoxystrobin has been detected in WI potato fields. A 2001 report documented *A. solani* partial resistance to azoxystrobin (F129L mutation). In our work, we have morphologically identified *A. solani* and *alternata* isolates with resistance to azoxystrobin as demonstrated by reduced germination on azoxystrobin-amended media. Eighty percent of our *A. alternata* isolates were completely resistant to azoxystrobin. Partial resistance to azoxystrobin was identified in a small subset of the *A. solani* isolates. Our further studies will confirm species through ITS sequencing and resistance through identification of mutation sites. Growers with azoxystrobin resistant *Alternaria* spp. isolates should avoid use of the fungicide in their disease management programs.

Histological characterization of wheat pre-and post-haustorial resistance components to the leaf rust pathogen *Puccinia triticina*

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Leaf rust caused by *Puccinia triticina* is the most prevalent disease of wheat worldwide. *P. triticina* is recognized as an important pathogen in wheat production worldwide causing significant yield losses over large geographical areas. Genetic resistance is the preferred method to reduce losses from leaf rust. Over 60 *Lr* genes have been designated but only a few are effective against the highly diverse North American *P. triticina* population. Histological examination of the plant-fungus interaction reveals pre-haustorial and post-haustorial resistance components. Previous research has shown that post-haustorial resistance tends to be involve in race specific resistance and is commonly characterized by presence of hypersensitive reaction (HR) while, pre-haustorial resistance do not generally involve a HR respond. The resistant mechanisms not based on hypersensitivity are presumed to be more durable and may be involved in broad and non-race specific resistance. In this study a set of near isogenic lines carrying different leaf rust resistance genes (*Lr* genes) were characterized for pre- and post-haustorial resistance using a single race inoculation in a time course experiment. Tissue samples from 24, 48 and 72 hpi were collected for histological analysis. Pathogen infection structure development and plant tissue responses, in compatible and incompatible interactions, were evaluated using fluorescence microscopy. Histological findings (microscopic phenotypes) were compared to disease infection type 14dpi (macroscopic phenotype). Preliminary results and implications for deployment of resistance genes will be discussed.

Assessing biological control agents and their fungicide sensitivities for potential integrated management of Sclerotinia stem rot in soybean

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Phytopathology 102(Suppl. 5):S5.4

Sclerotinia stem rot (*Sclerotinia sclerotiorum*) of soybean is a major problem in the North Central production area of the US. This study was conducted to evaluate the effects of biological and chemical strategies to manage the impact of stem rot disease in soybean. Three commercial biological control agents (BCAs): *Coniothyrium minitans* (Contans™ WG), *Streptomyces lydicus* (Actinogrow™ AG), *Trichoderma asperellum* and *Trichoderma gamsii* (Tenet™ WG) were applied to soil that was infested with sclerotia. *Coniothyrium minitans* was applied at different times [fall at 2 lb/a, spring at 2 lb/a, and (spring at 1 lb/a +fall at 1 lb/a)] combined with lactofen in a complete randomized split block design at two different locations. The results showed that *C. minitans* was the most effective in reducing the disease and lactofen enhanced its efficacy. In vitro, the highest percentage of colonization of *S. sclerotiorum* occurred from 15 to 20°C for *C. minitans* and 25°C for *Trichoderma* spp. A co-culture assay was conducted to evaluate the compatibility of biocontrol agents; *C. minitans*, *T. gamsii*, *T. asperellum*, and *S. lydicus* did not inhibit each other significantly on agar plates. The sensitivity of BCAs to tested chemicals was determined based on evaluating their growth on culture media amended with fungicides. *C. minitans* was not sensitive to tetraconazole, mefenoxam, and propamocarb hydrochloride; *T. gamsii* and *T. asperellum* were not affected by the fungicides mandipropamid, azoxystrobin, and boscalid at 1000 µg/ml. In conclusion, the tested BCAs can potentially be used to manage Sclerotinia stem rot individually or in selected combination, which will provide wider adaptability to different ecological niches and field conditions. Nevertheless some fungicides interaction with BCAs may affect their populations in treated soil.

Evaluation of *Rhizoctonia solani* - *Heterodera glycines* interactions on soybean

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Two soilborne pathogens of soybean, soybean cyst nematode (*Heterodera glycines*; SCN) and *Rhizoctonia solani*, co-infest fields in the U.S. To examine the interactive effects of these pathogens on soybean, field trials were conducted near Urbana, IL from 2009 to 2011. Combinations of a seed-applied fungicide (A16148) and nematicide (abamectin) were evaluated on SCN-susceptible and -resistant soybean cultivars. The entire field was naturally-infested with SCN, and soil of half the plots was artificially-infested with *R. solani*. Plant stand, yield, and SCN reproduction factor (Rf) were measured. In all three years, plant stand was significantly lower in plots infested with *R. solani* compared to non-infested plots, and stand was significantly improved in *R. solani*-infested plots when A16148 was applied to seeds. In 2009 and 2010, stand of the SCN-resistant cultivar generally was greater than stand of the SCN-susceptible cultivar, but this was not observed in 2011. In 2009, yields of the SCN-resistant cultivar were greater than yields of the susceptible cultivar in *R. solani*-infested plots, but not in the non-infested plots. In 2010 and 2011, yields of the SCN-susceptible and -resistant cultivars were similar except when abamectin was applied to the seed. In these instances, the SCN-resistant cultivar had a greater yield than the susceptible cultivar except in the plots not infested with *R. solani* in 2010. No trends of seed treatment effects on SCN Rf were observed across the three years. Based on our results, the performance of the SCN-susceptible cultivar often was less than the resistant cultivar, and the difference between the two cultivars often was greater in *R. solani*-infested plots. These results indicate that SCN-susceptible cultivars may be affected more severely by *R. solani* than SCN-resistant cultivars in SCN infested fields. To confirm these field results, greenhouse factorial experiments with *R. solani* and SCN are underway.

Broad spectrum virus resistance using direct repeat induced gene silencing (DRIGS)

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The RNA interference (RNAi) has been widely used to down regulate targeted transcripts and development of disease resistant plants. RNAi as a type of post-transcriptional gene silencing (PTGS) relies on the presence of dsRNA as an inducer for the entire silencing complex leading to the degradation/inactivation of the target mRNA. Here, we used a direct repeat induced gene silencing (DRIGS) vector as a tool for developing broad spectrum virus resistance in plants. Short fragments from different viruses were fused with a silencing locus in the DRIGS vector and transformed into *Arabidopsis*, *N. tobacco* and *N. benthamiana*. Virus inoculated transformed tobacco plants remain symptomless and no virus can be detected by ELISA. Virus specific siRNAs could also be readily detected in the transgenic plants. These preliminary results show that the system has the potential to provide broad spectrum resistance to multiple viruses simultaneously.

Monitoring *Pseudoperonospora cubensis* sporangia dynamics and population structure and testing fungicide efficacy to better control cucurbit downy mildew

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Cucurbit downy mildew has been a serious annual limitation to cucurbit production in the US since 2004. Prior to 2004, host resistance introduced in the 1950s and 1960s effectively limited cucurbit downy mildew in the US. To better understand *Pseudoperonospora cubensis* and downy mildew management, we investigated the seasonal and daily dynamics of airborne sporangia concentrations in cucurbit fields in Michigan, the global genetic structure of *P. cubensis* populations, and conducted fungicide efficacy trials in Michigan. Airborne sporangia concentrations were measured during the growing season in cucurbit fields in five Michigan counties from 2006 to 2009. Airborne sporangia were detected from June through September, and concentrations increased during this period. Peak airborne sporangial concentrations occurred between 0800 and 1300 h. Airborne sporangia concentrations were positively correlated with temperature and negatively correlated with relative humidity and leaf wetness. Six genetic clusters were identified using Bayesian clustering and seven genes in our sampling of 465 *P. cubensis* isolates from diverse countries and hosts. All of the genetic clusters were present in the sampling from North America and Europe, but differences in cluster occurrence were observed by country and state. Isolates

from cucumber had different cluster composition and lower genetic diversity than isolates from other cucurbits. Fungicide trials in 2009, 2010, and 2011 showed that several products are effective in limiting downy mildew when foliar application were applied on a 4- to 7-day schedule. Products may be used in rotation and tank mixed to improve efficacy and limit the development of resistant pathogen populations. Implications of these results for managing cucurbit downy mildew will be discussed.

Soybean race-specific resistance *Rps3a* and *Rps8* to *Phytophthora sojae* are not allelic

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Phytophthora sojae is a major soil-borne pathogen of cultivated soybean in regions with poorly drained soils. Susceptible interactions to *P. sojae* occur at all stages of soybean development, resulting in rotting of seeds, root and stem rot, as well as yield reduction due to the decrease in number of plants. To date, the deployment of soybean varieties with race-specific resistance has been the primary management strategy against *P. sojae*. *Rps8* is a resistance gene to *P. sojae* originating from soybean PI 399073. Previous studies localized *Rps8* to chromosome 13. Another resistance gene to *P. sojae* with three known alleles have been previously assigned to chromosome 13 and are designated *Rps3a*, *Rps3b* and *Rps3c* respectively. In this study, sources of *Rps3a* and *Rps8* were tested for their response following inoculation with isolates of *P. sojae* via the rolled towel hypocotyl inoculation assay. Sources of *Rps8*, including PI 399073 and select lines from BC₄F_{4.5} Williams x PI 399073 (cv. Williams as recurrent parent), and sources of *Rps3a*, -3b, and -3c responded differentially following inoculation with 40 different isolates of *P. sojae*. Allelism test between *Rps8* and *Rps3a* in an F_{3:4} L83-570 (*Rps3a*) x PI 399073 population of 319 lines with isolate OH25 (vir 1a, 1b, 1c, 1k, 7) suggests the two genes are separate but linked within the soybean genome. Based on the differential response following inoculation with isolates of *P. sojae* and the segregation ratios in the F_{3:4} populations, *Rps8* is linked but not allelic to *Rps3a*.

Variability in postharvest rot response of sugar beet (*Beta vulgaris*) recombinant inbred lines

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Sugar beet (*Beta vulgaris*) is commonly harvested and stored in outdoor piles prior to processing for food and animal feed. During this storage period the crop is subject to multiple post-harvest rots. In recent survey work in the Michigan growing region, several fungi have been identified that are known to cause severe post harvest rot. Resistance to three post harvest rots was identified in two sugar beet germplasm in the 1970s, but there has been little work done on host resistance to post-harvest storage pathogens in recent years. Recombinant inbred lines of sugar beet have recently been developed, and these were screened for susceptibility to biotic post-harvest deterioration. Significant differences ($P < 0.05$) were found in this population for responses to three pathogens: *Botrytis cinerea*, *Penicillium claviforme*, and *Rhizopus stolonifer*. The poor correlation between reduced damage by these pathogens suggests independent genetic control of susceptibility. There is the potential to develop materials that may be less damaged by post harvest rot pathogens for the North Central US growing region, as well as gaining a better understanding of the interaction between fungal storage rot pathogens and host genotype.

Using association analysis to detect regions of the genome that confer resistance to Fusarium head blight in eastern U.S. soft red winter wheat

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Fusarium head blight (FHB) (*F. graminearum*) is one of the most serious diseases of wheat and other cereal crops. Infection occurs during flowering and early grain fill under wet, humid conditions. Damaged kernels are shrunken and discolored, leading to yield reduction and poor grain quality. Infection also leads to contamination with deoxynivalenol (DON), a mycotoxin produced by *F. graminearum*, that makes the grain unfit for consumption. Resistance to FHB is a quantitatively inherited trait controlled by multiple genes and can be confounded with other agronomic traits. An association analysis aims to detect linkage between markers and genes

controlling resistance in complex populations. The objective of this study was to determine the magnitude and location of QTLs associated with FHB resistance in eastern U.S. soft red winter wheat. A population of 400 lines was genotyped with 1,820 DArT markers. FHB index was determined in 2010 and 2011 as the percentage of spikelets showing symptoms, with three replicates per year. A mixed linear model for DArT FHB association was performed using TASSEL 3.0. Thirty-two QTL on 18 chromosomes were found to be significantly ($P < 0.05$) associated with FHB resistance. Two of these QTL were also found to be associated with heading date. Most QTL identified explained less than 2.2% of the variance. A QTL on chromosome 7B decreased FHB by 4.36% and the frequency of the allele conferring resistance was 0.05. Another QTL on chromosome 4B decreased FHB by 4.24% and the frequency of the resistant allele was 0.94. For most of the identified QTL, the frequency of the allele conferring resistance in this population is low, indicating that resistance could be increased by fixing alleles. Since the allele effects are small, genomic selection may offer the best strategy to combine the additive effects of small QTLs.

Genomic characterization of the conditionally dispensable chromosome in *Alternaria arborescens* provides evidence for horizontal gene transfer

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Fungal plant pathogens cause serious agricultural losses worldwide. *Alternaria arborescens* is a major pathogen of tomato, with its virulence determined by the presence of a conditionally dispensable chromosome (CDC) carrying host-specific toxin genes. Genes encoding these toxins are well-studied, however the genomic content and organization of the CDC is not known. To gain a richer understanding of the molecular determinants of virulence and the evolution of pathogenicity, we performed whole genome sequencing of *A. arborescens*. Here we present the de-novo assembly of the CDC and its predicted gene content. Also presented is hybridization data validating the CDC assembly. Predicted genes were functionally annotated through BLAST. Gene ontology terms were assigned, and conserved domains were identified. Differences in nucleotide usage were found between CDC genes and those on the essential chromosome (EC), including GC3-content, codon usage bias, and repeat region load. Genes carrying PKS and NRPS domains were identified in clusters on the CDC and evidence supporting the origin of the CDC through horizontal transfer from an unrelated fungus was found. We provide evidence supporting the hypothesis that the CDC in *A. arborescens* was acquired through horizontal transfer, likely from an unrelated fungus. We also identified several predicted CDC genes under positive selection that may serve as candidate virulence factors.

Relationship between the greenhouse and field reactions of spring wheat genotypes to Bacterial leaf streak pathogen (*Xanthomonas campestris* pv. *translucens*)

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Bacterial leaf streak caused by *Xanthomonas campestris* pv. *translucens* has recently become a disease of growing concern for spring and winter wheat production in the U.S.A. Field evaluation of germplasm can be complicated due to environmental variation and mixed infection with other diseases. The objective of this study was to examine relationships among reactions observed in field and greenhouse screenings to help develop a reliable germplasm screening method. Field experiments were conducted in Brookings and Codington counties, SD during 2009 and 2010 and a greenhouse test was completed in 2010. Bacterial suspensions with concentrations of 3×10^8 CFU per ml were inoculated onto forty-five hard red spring wheat genotypes in field trials. A subset of twenty highly diseased to moderately-resistant genotypes were selected and tested in the greenhouse using inoculation methods similar to field tests. Results showed significant differences among genotypes for disease severity in all trials. Genotypes SD4205 and SD4148 consistently had low level of disease compared to other genotypes across all evaluations. The response of genotypes in greenhouse and field evaluations were comparable and significant positive correlations were observed for disease severity, as reflected by area under disease progress curve (field test) and percent diseased-leaf-area (greenhouse test). These results indicate that genotypes can be successfully evaluated in either setting with artificial pathogen inoculation. Findings of this research should be useful for germplasm evaluation leading to selection and development of moderately-resistant cultivars.

Factors involved in Indiana bitter rot outbreaks

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Bitter rot, caused by *Colletotrichum acutatum* and *C. gloeosporioides*, is a significant late-season disease of commercial apples. We identified four factors implicated in the Indiana bitter rot outbreaks of 2010 and 2011: cultivar susceptibility, weather conditions, fungicide spray schedules and fungicide resistance. Summer temperatures and rainfall exceeded the 30-year average by at least 1 degree Celsius and 2.5 cm, respectively, in both years. Review of spray records found that growers with losses to bitter rot did not use captan at the maximum-labeled rate and extended applications to the longest labeled interval. Isolates were obtained from fruit collected from three Indiana apple orchards to test whether fungicide resistance played a role in this outbreak. Thirty-five isolates from each orchard were screened for resistance to the fungicides kresoxim-methyl and thiophanate-methyl. Preliminary data suggest that 20% of the isolates were resistant at 0.2 ppm kresoxim-methyl, and 30% of all isolates were shifted in resistance. In 2 of the 3 orchards, 100% of the isolates were resistant to thiophanate-methyl; in the third, only 3% tested as resistant. These findings suggest that growers should modify their bitter rot management practices during seasons that are unusually hot and wet by using the maximum rate and shortest interval when applying captan while also recognizing the risk of resistance to strobilurin fungicides.

Factors affecting the development and severity of *Clavibacter michiganensis* subsp. *nebraskensis* of *Zea mays*

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Goss's bacterial wilt and blight (*Clavibacter michiganensis* subsp. *nebraskensis*) of corn (*Zea mays*) recently reemerged and is becoming increasingly important in corn fields of North America. The objective of this study was to identify and prioritize both environmental and agronomic factors contributing to the development and progression of Goss's wilt across the Corn Belt. To identify which factors contributed to disease development, a regional survey was conducted to identify the production practices and growing conditions during 2011. A total of 486 surveys and plant samples were submitted as part of the project in 2011, with 146 (30%) samples testing negative for the Goss's wilt pathogen and 340 (70%) testing positive. Results were analyzed with two methods in the R statistical program, including Classification and Regression Tree Analysis (CART) and Random Forest. Results from both CART and Random Forest were used to identify and prioritize contributing factors. A chi square test of homogeneity was also used to analyze portions of the dataset. Results indicated that the hybrid Goss's wilt ratings (assigned by the seed company), followed by planting population, crop rotation, planting date, and residue cover were the five most important factors determining Goss's wilt development during 2011. Results of the survey indicated that a number of factors, including planting susceptible hybrids, high planting populations, and planting continuous corn could be responsible for disease development and progression. Data from this survey will help focus research efforts for improved management recommendations for Goss's bacterial wilt and blight by highlighting the most influential factors affecting Goss's wilt development.

Four common foxtail species are alternate hosts for *Clavibacter michiganensis* subsp. *nebraskensis*, causal agent of Goss's bacterial wilt and blight of *Zea mays*

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Goss's bacterial wilt and blight, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn) has reemerged as an important disease of *Zea mays* (corn) in the Midwest since approximately 2006. Results from a 2011 multi-state survey indicated that *Setaria* spp. (foxtail) were commonly present in corn fields with a history of Goss's wilt. The objective of this research was to test *Setaria* spp. that are common in the Midwest for their susceptibility to Cmn. In the greenhouse, seedlings of four *Setaria* spp. including *S. viridis*, *S. faberii*, *S. verticillata*, *S. glauca*, and Golden Cross Bantam sweet corn were inoculated with a suspension of 1.0×10^7 bacteria cells. A mixture of four isolates with demonstrated virulence during previous testing was used. The trial was arranged in a randomized complete block design and repeated one

additional time. Percent of symptomatic leaf area affected was visually estimated eight days after inoculation. Bacterial streaming was confirmed microscopically and Cmn was re-isolated from all four species. *S. faberii* (giant foxtail) was the most susceptible in both trials having 22% and 18% leaf area affected in trials 1 and 2, respectively. *S. viridis* (green foxtail) was the next most susceptible having 12% and 10% leaf area affected, respectively. *S. verticillata* (bristly foxtail) was also susceptible having 8% and 5% leaf area affected followed by *S. glauca* (yellow foxtail), which was the least susceptible with 5% and <1% leaf area affected, respectively. Results indicate that these four *Setaria* spp. are susceptible to Cmn and could serve as an important alternate host and potentially reduce the effectiveness of crop rotation to manage Goss's bacterial wilt and blight.

Identification and comparison of quantitative trait loci conferring partial resistance to *Phytophthora sojae* in PI 427106 and PI 427105B originating from China

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Phytophthora root and stem rot caused by *Phytophthora sojae* is an economically damaging disease of soybean in the North Central region. Single-gene (*Rps*) resistance provides an effective defense to this disease. However, rapid adaptation by the pathogen emphasized the use of partial resistance. Partial resistance is effective to many pathotypes of *P. sojae* and believed to be durable resistance. The objectives were i) to identify quantitative trait loci (QTL) for partial resistance to *P. sojae* isolate 1.S.1.1 in two recombinant inbred populations, and ii) to compare and contrast the QTL between two populations. The populations were derived from OX20-8 (susceptible) crossed with PI 427106 or PI 427105B, both originated in China and with high level of partial resistance. The populations were genotyped with approximately 200 single nucleotide polymorphism markers and genetic maps were constructed separately. Levels of partial resistance were evaluated by layer tests, where root rot, plant weight, root weight, and dry root weight were measured for the disease phenotypes. Seven QTL were identified in each population, three of them were common in both populations. Of the three, one major QTL on chromosome 18 explained 36% of phenotypic variance, while the remaining two accounted for 2 to 8% of the variance. Two germplasm accessions from China are potential new sources to improve partial resistance to *P. sojae* in soybean.

5' untranslated region and the 58 kilodalton protein of Bean pod mottle virus RNA2 are essential for RNA2 replication

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Bean pod mottle virus (BPMV) is a positive (+) sense, single stranded RNA virus with two separately encapsidated genomic RNAs: RNA1 and RNA2. RNA1 encodes the viral proteins required for viral RNA replication. Conversely, RNA2 encodes a 5' proximal 58 kilodalton (K) protein with as-yet-unknown function, a 48 K movement protein that is co-terminal with the 58K protein at its C-terminus, and two coat protein subunits. RNA1 can replicate by itself, whereas RNA2 can only replicate in the presence of RNA1. To further determine whether RNA2 contains specific RNA elements or protein products that are essential for its own replication, we have generated chimeric RNA2 constructs with either 5', or 3', or both untranslated regions (UTRs) replaced by their RNA1 counterparts. In addition, we also produced two RNA2 mutants in which the translation of either 58K or 48K was disrupted so that only one of the two proteins would be produced. To test the replicational competence of these mutants, the mutant cDNAs were introduced into lima bean cotyledons together with the cDNA of RNA1, with a particle bombardment procedure. We found that the chimeric RNA2 construct with RNA1 3'UTR replicated efficiently and was capable of systemic movement in host plants, where the one with the 5'UTR of RNA1 did not. Furthermore, RNA2 mutants with the 58K translation disrupted also failed to replicate. In summary, our results indicate that the 5'UTR of RNA2 together with the 58K protein are required for RNA2 replication.

Developing penthiopyrad for controlling *Rhizoctonia solani* in sugarbeet

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Rhizoctonia solani, a soil borne fungus, causes damping-off, crown rot and root rot of sugarbeet (*Beta vulgaris* L.). Sugarbeet varieties resistant to *R. solani* and high yielding are difficult to develop. Cultural practices including early planting into cool soils, crop rotation with non-hosts such as wheat and barley, use of partially resistant varieties and the use of effective fungicides

are necessary to manage *R. solani*. Azoxystrobin, a systemic fungicide and a quinone outside inhibitor, is the most effective and widely used fungicide for controlling *R. solani*. It will be useful to have other effective fungicides with different modes of action. The objective of this research was to evaluate penthiopyrad for its efficacy at controlling *R. solani*. Penthiopyrad, a systemic fungicide, employs the succinate dehydrogenase inhibitor (SDHI) mode of action to suppress pathogens. Penthiopyrad was evaluated in greenhouse and field studies for its utility as a seed treatment at 14 g/unit seed and as an in-furrow application at 2.8 litre ha⁻¹. In the field, 10-34-0 starter fertilizer at 28 litre ha⁻¹ was added to the fungicide treatments since this is a common practice by growers. Penthiopyrad as a seed treatment and in-furrow provided significantly greater plant stand and higher recoverable sucrose compared to the non-treated and starter fertilizer checks, and similar yields to the standard azoxystrobin in-furrow application. Penthiopyrad was evaluated in the greenhouse applied in-furrow at rates of 1.1, 1.5, 2.2 and 2.8 litre ha⁻¹ before plants were inoculated with *R. solani* grown on barley. Penthiopyrad at all rates provided significantly greater control than the non-treated check. However, penthiopyrad at 1.5 and 2.2 litre ha⁻¹ provided similar control as the non-inoculated check. These trials demonstrate that penthiopyrad has the potential to be used for control of *R. solani* and as a fungicide resistance management tool.

Characterization of the causal agent of bacterial leaf spot in the Midwest U.S.

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Bacterial leaf spot (BLS) disease of tomato is a major threat to the processing tomato industry in the U.S. Midwest. Recently, tomato fruits infected with BLS were found to develop unusually large, deep, cracked lesions that resist removal by peeling during processing. *Xanthomonas* strains were isolated from symptomatic tomato fruit and leaf tissues collected in processing tomato production fields in OH, MI and IN in 2010 and 2011. A suite of Polymerase Chain Reaction assays with different primer sets (RST65/69, RST27/29, J19/22 and BOXA1R) were conducted with isolated strains to determine their species identities. In the 2010 survey, 75% of the 83 recovered *Xanthomonas* strains were *X. gardneri*, 13% were *X. euvesicatoria*, and 10% were *X. perforans* (Plant Dis. 95:1584). Two strains could not be identified to species. In the 2011 survey, 60% of the 82 recovered *Xanthomonas* strains were identified as *X. gardneri*, 1% were *X. euvesicatoria*, and the remaining 39% were *X. perforans*. This is the first time a transition of the majority of causal organisms of BLS in the Midwest US from *X. euvesicatoria* to *X. gardneri* has been documented. The efficacy of Kocide 3000, Cuprofix, Actigard, Kathon and Kocide 3000 + Manzate, with or without the surfactant Activator 90, in reducing BLS incidence and severity in processing tomatoes (cv. Heinz 3402) was evaluated in 2011. Disease pressure was high and none of the products significantly reduced BLS compared to the non-treated control. However, the incidence of BLS-infect fruit was significantly higher (12.3%, $P = 0.023$) in plots treated with Kocide 3000 + Activator 90 than in the non-treated control (6.6%).

Studies on the effect of corn hybrid and foliar fungicide on disease severity and yield in corn in the U.S. Corn Belt

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Hybrid corn is an economically important crop for the U.S., the world's leading producer. Corn is susceptible to several foliar fungal diseases, including gray leaf spot caused by *Cercospora zeae-maydis* and northern leaf blight, caused by *Exserohilum turcicum*. Cultural methods like planting resistant hybrids and reducing infected debris by rotation and tillage have been used for disease management in hybrid corn. Increasingly though, the use of foliar fungicides has shifted away from seed and specialty crop production to be applied on millions of acres of hybrid corn. Several potential factors have been used to justify this increased use, including, higher incidence of foliar diseases, higher commodity prices for corn, and more fungicides available and marketed for yield benefit beyond just disease management. Field research studies were conducted from 2010 to 2011 in Iowa, Wisconsin, Ohio and Illinois to examine the effect of corn hybrid, foliar fungicide, and disease intensity on corn yield. These field studies are a part of a larger goal to standardize disease management recommendations and develop a regional corn fungicide resistance monitoring program. An overall goal is to determine if there is an economical foliar fungicide spray threshold for foliar diseases of corn based on spray thresholds developed using yield

loss models. Preliminary results from these multi-state studies will be presented along with current recommendations for managing foliar diseases of corn in the north central region.

The HrpL regulon in *Erwinia amylovora* includes novel virulence factors unrelated to type three secretion

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Erwinia amylovora is the causative agent of fire blight, a disease apple and pear. Multiple pathogenicity factors have been identified in *E. amylovora* including the type III secretion system (T3SS). HrpL, a transcription factor, coordinates the expression of all components of the T3SS via the *hrp* promoter and is required for fire blight development. The role of HrpL in T3SS regulation does not preclude the possibility that HrpL may also regulate other virulence factors in *E. amylovora*. To explore the HrpL regulon in *E. amylovora* Ea1189, wild-type and Ea1189Δ*hrpL* transcriptomes were compared *in vitro* with microarray analysis and novel *hrp* promoters were identified *in silico* using hidden Markov modeling. Using this combinatorial approach, 51 genes were identified as differentially regulated by HrpL. Of these, 28 do not have predicted roles in T3SS and may represent novel virulence factors. To explore putative virulence roles, eight deletion mutants were generated in *E. amylovora* Ea1189. Mutants were screened for virulence phenotypes in immature pear fruit, hypersensitive response (HR) in tobacco, as well as biofilm formation and motility *in vitro*. Six mutant strains were significantly reduced in virulence and, to date, three mutants were confirmed to not affect the HR in tobacco suggesting virulence functions unrelated to T3SS. Continued analysis of the non-T3SS HrpL regulon will contribute to a complete understanding of the pathogenicity of *E. amylovora*.

Distribution and titer of Rice black-streaked dwarf virus in maize

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Rice black-streaked dwarf virus (RBSDV) is a planthopper-transmitted fijivirus that causes an important disease of maize (*Zea mays* L.) in China. Research on the virus has been limited by difficulties with transmitting and maintaining the virus in plants. We investigated parameters important for mechanical transmission of RBSDV to maize kernels with vascular puncture inoculation (VPI), and the subsequent distribution and titer of the virus in infected plants. Transmission was evaluated by symptom development beginning 10 days post-inoculation (dpi) and Protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA) was used to determine the relative virus concentration in plant tissues. Under greenhouse conditions in Ohio, the first symptoms of RBSDV infection appeared at 19 dpi as 1–2 mm chlorotic streaks on the 5th leaf, followed by development of swollen tertiary veins, and enations on the ventral surface of the leaf. Later emerging leaves were dark green and the plants were severely stunted, as is typical of fijivirus infection. Virus titer in the youngest, expanded leaf was examined between 19 and 68 dpi using PAS-ELISA. Titer was similarly high in the tissue between 26 and 61 dpi, with significantly lower titer at 19 and 68 dpi. The distribution of RBSDV in roots and the 13 emerged leaves was examined at 41 dpi. Virus titers were high in leaves 5–11 and fibrous roots, and were lower in the youngest emerging leaves, oldest senescing leaves and brace roots. These findings will help to define disease progression in maize, and identify the best tissues for virus inoculum preparation.

Determining the best time to use azoxystrobin for controlling *R. solani* in sugarbeet

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Rhizoctonia root and crown rot of sugarbeet (*Beta vulgaris* L.) is the worst production problem for growers in North Dakota and Minnesota. The causal organism is the fungus *Rhizoctonia solani* Kühn. Azoxystrobin provides effective control against *R. solani*, but it must be applied just prior to infection. Previous study showed that *R. solani* infects sugar beet when the average daily soil temperature at the 10 cm depth is 18° C or higher. However, American Crystal Sugar Company recommends that azoxystrobin should be applied when sugarbeet are at the 4–6 leaf stages or larger. The objective of this research was to determine whether azoxystrobin provides protection to sugar beet seeds and seedlings younger than 4 leaf stage from *R. solani* at temperature ≥18° C. Research was conducted in the greenhouse at 21

$\pm 2^{\circ}\text{C}$. Azoxystrobin was applied at 0.672 L/ha in-furrow, and in 18 cm band applications on seeds, cotyledons, two-leaf and four-leaf sugarbeet followed by inoculation with *R. solani* AG 2-2 III B grown on sterilized barley grain. Inoculated and non-inoculated checks were included for all growth stages. The inoculated check resulted in death of all seedlings or plants indicating that the inoculum was effective. Non-inoculated checks resulted in healthy plants. Azoxystrobin provided effective control against *R. solani* at all growth stages when applied in-furrow and in the band applications. This data indicate that 4 leaf and younger sugarbeet plants are infected by *R. solani* under favorable environmental conditions. Consequently, azoxystrobin should be applied to control *R. solani* in sugarbeet before the threshold of 18°C is reached in the presence of adequate moisture, irrespective of growth stage.

Reduced sensitivity of the cherry leaf spot fungus *Blumeriella jaapii* to the succinate dehydrogenase inhibitor fungicide boscalid

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Cherry leaf spot (CLS), caused by the fungus *Blumeriella jaapii*, is a major disease of tart (*Prunus cerasus*) cherry trees. Leaf spot can lead to early defoliation if left untreated which can result in poor fruit quality, reduced fruit set, and in severe cases death of the tree. The most common tart cherry cultivar Montmorency, requires yearly chemical control programs, and reliance on site-specific fungicides for management increases selection for resistance in fungal populations. One commonly-utilized fungicide for CLS management in Michigan is Pristine, a premix of boscalid and pyraclostrobin. These site-specific materials have a high risk for developing resistance within fungal populations. The focus of this study was to assess the sensitivity of *B. jaapii* populations to boscalid. A total of 327 Isolates from commercial orchards in Michigan and 44 isolates from non-treated trees in Ohio were collected in 2010 and assayed. Single spore isolates were grown on fungicide-amended media at concentrations of 0, 0.1, 0.5, 1, 2.5, 5, 10, and 25 $\mu\text{g/ml}$. The minimum inhibitory concentration (MIC) of boscalid for each isolate was determined after incubation at 23°C for 14 days. Isolates from non-treated trees were sensitive to boscalid with MIC values ranging from 0.1 to 0.5 $\mu\text{g/ml}$. Isolates from several commercial orchards showed reduced sensitivity with MIC values ranging from 1 to >25 $\mu\text{g/ml}$. More than 60% of isolates from two orchards were not sensitive at the highest concentration of 25 $\mu\text{g/ml}$. Our results indicate that *B. jaapii* populations may be shifting towards reduced sensitivity to boscalid and further surveys are needed to assess the occurrence of resistance in Michigan *B. jaapii* populations.

Survey of oomycete species associated with soybean seedling diseases in the U.S.

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Soybean is the second most important crop in the U.S., but yield is often reduced by diseases affecting stand establishment and root health. Oomycete pathogens have been commonly associated with seedling damping off and root rots, particularly under conditions of excessive moisture. However, there is limited information regarding their frequency and abundance across the major soybean producing states in the Midwest. In order to determine the distribution and prevalence of oomycete species associated with soybean seedling diseases, a survey was conducted across 12 states. Nearly, 300 oomycete isolates were recovered from five different fields in each state, using semi-selective medium (CMA-PARP+B). The resulting isolates were identified by sequencing of the ITS region of rDNA. Preliminary sequence results distinguished a total of 54 *Pythium* and 2 *Phytophthora* spp., *Py. sylvaticum* (15%) and *Py. oopapillum* (18%) being the most frequent; even so, species composition frequency fluctuates greatly between sampling locations, revealing putative spatial gradients. Pathogenicity, aggressiveness, and morphological identification are currently being determined for representatives of each species. The distribution, and frequency of the species found in this study provide valuable information on abundance and prevalence of potential soybean oomycete pathogens across different geographical locations and conditions. This study will provide a foundation for the development of diagnostic tools, the facilitation of improved soybean breeding, and management of seedling root health.

Timing the efficacy of fungicide applications for *Diplodia* ear rot management

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Stenocarpella maydis is one of the most prevalent ear rot pathogens in corn fields. The absence of durable commercial resistance makes it necessary to explore alternative options for management. Currently, one fungicide is labeled for use on this disease: Quilt Xcel (azoxystrobin + propiconazole; Syngenta Crop Protection). A field experiment was conducted near Lafayette, IN, in 2011 to determine efficacy of Quilt Xcel on *Diplodia* ear rot in a susceptible hybrid. The experiment was designed as a split-plot with inoculation treatment as the whole plot, and fungicide application timing as the subplot. Plants receiving an inoculation treatment were inoculated by placing *S. maydis* colonized sorghum in the whorl during vegetative growth stages (V7). Fungicide treatments were randomly assigned within each whole plot, and consisted of an application of the label rate of fungicide at an early vegetative growth stage (V6), tasseling/silking (R1) or milk stage (R3). An untreated control was also included in each inoculation treatment. Disease incidence and severity were scored at plant maturity. Seed weight and seed moisture were measured, and converted to bushels per acre to determine yield. Fungicide treatment did not significantly reduce disease severity at any application timing compared to the untreated control in non-inoculated or inoculated plots. Fungicide treatment did not have a significant effect on yield.

Development and application of a qPCR assay to track the spread of a microbial biopesticide

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Cryptococcus flavescentis strain OH182.9_3C (3C) is a basidiomycete yeast that was previously shown to have biocontrol activities against Fusarium head blight of wheat. A qPCR assay was developed to track the natural spreading of 3C in the field. Briefly, potential target genes for qPCR amplification were identified through genomic analysis of 3C, and primers for SYBR Green qPCR were designed based on the identified target genes. The qPCR assay was validated and optimized using representative samples, and then applied to quantify the 3C colonization levels of the wheat head samples collected at different time points from the field where a few plots were inoculated with 3C. The qPCR results showed that 3C were progressively spread from artificially inoculated plots to non-inoculated plots as time passed, and that the spreading patterns were stochastic temporally and spatially. This research will provide insights into the ecological behaviors of 3C in the field and information necessary for the EPA registration of 3C as a biopesticide.

A cost-benefit assessment of integrated management strategies for Fusarium head blight and deoxynivalenol control in wheat

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When conditions for Fusarium head blight (FHB) development and deoxynivalenol (DON) contamination of wheat grain are favorable, substantial reductions in grain yield and quality may occur. Components of FHB-related losses include lower grain yield, price discounts due to DON contamination, Fusarium damaged kernels (FDK), and low test weight, as well as costs associated with disease management. These all have a negative effect of gross cash income (GCI). Growers are advised to implement management strategies such as tillage, crop rotation, cultivar resistance, and timely fungicide application, as well as grain harvesting strategies to minimize losses due to FHB and DON. However, there are costs associated with these strategies that are not always offset by gains in grain yield and quality. For instance, modifying combine harvester configurations to remove diseased kernels during harvest improves grain quality, but the benefit of this approach depends on baseline yield, FHB, and DON levels and grain prices. Similarly, the benefit of applying a fungicide is not always sufficient to offset application cost. Both fungicide application and grain harvesting strategies may reduce grain price discounts, and consequently increase GCI, as a result of lower FDK and DON, and higher test weights. Results of cost-benefit analyses of FHB and DON management strategies will be discussed for a range of baseline grain yields and FHB and DON levels, fungicide application costs, grain prices, and price discounts. Relationships among grain yield, FHB, DON, FDK, and test weight, as influenced by fungicide application, cultivar resistance, and grain harvesting strategies will be used as the basis for these analyses.

Assessing fitness of new *Phytophthora infestans* clonal lineages by comparing sporangial production on tomato leaves

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Phytophthora infestans, the oomycetous pathogen causing late blight of potato and tomato, is a major cause of crop losses worldwide. Three new pathogen clonal lineages, US-22, US-23, and US-24 have recently been identified in the U.S. To improve our understanding of fitness of the new lineages, we quantified sporangial production of each on detached tomato leaves. 'Brandywine Red' leaf disks were inoculated with sporangial suspension of a representative isolate of each lineage and incubated for 9 days. We evaluated the technique of sporangial harvest by comparing extraction with water alone and water with the surfactant Tween 20. Sporangial suspensions from 15-mm leaf disks were quantified using a haemocytometer and sporangia/cm² were calculated. The addition of Tween 20 resulted in significantly higher sporangial counts for US-22 ($p = 0.01$) and US-23 ($p < 0.001$), but not for US-24 ($p = 0.236$) when compared to the non-Tween 20 extraction method. With the addition of Tween 20, sporangial counts between US-22, US-23, and US-24 were significantly different (US-23>US-22>US-24); without Tween 20, counts were not significantly different between lineages. Results indicated that significant differences between sporangial counts by the different clonal lineages may be overlooked without the use of Tween 20. The significantly greater sporangial production by US-23 on tomato leaves suggested increased fitness of this lineage over US-22 and US-24. This work demonstrated that new *P. infestans* clonal lineages differ in sporangial production on tomato leaves and that the use of the surfactant Tween 20 is important in elucidating this difference.

Impact of temperature on growth and development of Northern and Southern isolates of *Macrophomina phaseolina*

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The fungal pathogen, *Macrophomina phaseolina*, causes charcoal rot on a wide variety of plants. Associated with periods of hot and dry weather, this disease mainly impacts soybean production in the southern US. However, there has been a recent increase in the incidence and severity of charcoal rot in northern soybean producing states, which may be the result of changing weather patterns. The purpose of this experiment was to compare several different isolates of *M. phaseolina* from the northern and southern US to determine the impact of temperature on growth and microsclerotial development. Six isolates, three from each region, were plated on potato dextrose agar (PDA) and soybean seed agar (SBA), and placed in an incubator at 15, 20, 25, 30, 35, and 38°C. Measurements of colony diameter (mm) and microsclerotial density (MSD, in microsclerotia per 12.5 mm²) were recorded daily for each isolate, for seven days. Results indicate there was little difference in growth between isolates at 30 and 35°C. However, southern isolates had a greater area under the growth progress curve (AUGPC) than northern isolates at 20 and 25°C, while northern isolates had a greater AUGPC than southern isolates at 15 and 38°C. MSD was greater for southern isolates than northern isolates at 25, 30, 35, and 38°C. This indicates that southern isolates are more capable of forming infective structures at warmer temperatures. No isolate produced mature microsclerotia at 15°C. All isolates grew more vigorously on PDA (387.1 AUGPC) than SBA (317.7 AUGPC) regardless of temperature. These results indicate that there are potential differences in the behavior of isolates from different regions at different temperatures. Additional research is needed to determine if these differences effect isolate virulence.

Austrian pine phenolics, likely contributors to systemically induced resistance to *Diplodia* canker

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Plants produce a vast array of specialized metabolites with no identified role in primary plant metabolism. However, it has long been hypothesized that many of these compounds are involved in plant defense. Indeed, several compounds act as feeding deterrents against animals, have direct toxic properties against microbes, and protect plants from environmental stress. The systemic induction of chemical defenses is believed to endow some plants with enhanced resistance to further pathogenic attack, a phenomenon known as systemic induced resistance (SIR). In our study, we examined the antifungal properties of several previously identified phenolic biomarkers of Austrian pine resistance to the needle blight and canker pathogen *Diplodia pinea*. Compounds were tested at concentrations detected in *planta*, from tissues exhibiting the SIR phenotype, using *in vitro* bioassays. We found that all compounds tested exhibited some antifungal properties, but the polyphenol polymer lignin had the greatest individual antifungal activity. Fungistatic

growth inhibition was achieved when compounds were tested as clusters (combinations) based on their coordinated metabolic regulation. Significant differences in growth inhibition between constitutive and induced concentrations of individual compounds and the clusters were observed, indicating that the examined phenolics likely play a key role in the expression of the SIR phenotype. This study exemplifies the value of evaluating potential biomarkers of resistance at *in planta* concentrations and the importance of co-regulation of chemical defenses in establishing resistance.

The influence of parasitic interactions on host gene silencing

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Many of the plant pathogens responsible for reducing crop yields have a biotrophic lifestyle, yet much remains unclear about how these parasites succeed at draining resources from their host. One such parasite, root-knot nematode (RKN, *Meloidogyne* spp.), establishes intimate feeding sites (giant cells) within the roots of a variety of plant hosts. Microarray data generated from laser-captured giant cells in *Arabidopsis thaliana* roots suggest that the RKN infection process may be influencing mechanisms in the host's gene silencing pathways. Subsets of genes regulated by trans-acting small RNAs and micro-RNAs, as well as the expression of several silencing machinery components, are upregulated during the nematode infection process. Preliminary results examining the effects of compromising these silencing pathways using knock-out mutants in *A. thaliana*, also suggest these components influence the host's susceptibility to RKN infection. Other lines of evidence suggesting disruption (or suppression) of these pathways have been generated using multiple transgenic tobacco lines that contain a silenced reporter gene. In addition to RKN, soybean cyst nematode (SCN, *Heterodera glycines*), and the parasitic plant dodder (*Cuscuta pentagonia*), all trigger restoration of the reporter gene during their biotrophic interactions. Confirmed involvement of host gene silencing with the nematode parasitism process would be noteworthy as it would heighten our understanding of the roles and connections between silencing and defense response pathways, and provide insight into how we may incorporate RNA interference in the control of biotrophic pathogens.

The role of a velvet-like complex in fungal development and virulence of the fungal cereal pathogen *Cochliobolus sativus*

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Cochliobolus sativus (Anamorph: *Bipolaris sorokiniana*) causes several important diseases, including spot blotch, black point and common root rot, in barley and wheat. The fungus produces typical necrosis and chlorosis on leaves of susceptible barley hosts and therefore host-selective toxins (HSTs) are hypothesized to be involved in the virulence. Our recent study suggested that non-ribosomal peptide synthetases are required for the biosynthesis of HSTs in *C. sativus*. However, the global regulation of biosynthesis of the HSTs or secondary metabolites is not well understood in this fungus. Recently, the velvet complex containing LaeA, VeA, and VelB have been shown to be involved in global regulation of secondary metabolism and/or fungal development in *Aspergillus nidulans* and several other fungal pathogens. To characterize the function of a velvet-like complex in *C. sativus*, orthologues of the three component genes (CsVeA, CsVelB and CsLaeA) were identified from the genome sequence of the fungus. Single gene deletion mutants (Δ CsVeA, Δ CsVelB and Δ CsLaeA) and double gene deletion mutants (Δ CsVelB+ Δ CsVeA, Δ CsLaeA+ Δ CsVeA and Δ CsLaeA+ Δ CsVelB) were generated and characterized. Our data indicated that these mutants altered to various extents in spore size, spore productivity, sensitivity to oxidative stress, as well as aerial mycelial growth depending on growth conditions as compared to the wild type strain ND90Pr. The Δ CsVeA and Δ CsVelB strains were significantly reduced in virulence while the virulence reduction was slight for the Δ CsLaeA strains as compared to the wild type. Among all the mutants, the Δ CsVelB+ Δ CsVeA strain was reduced the most in virulence. However, no significant differences were observed between the wild type and the mutants in spore germination rate and infection structure on barley leaves inoculated. This information will facilitate our understanding of the molecular mechanisms underlying fungal development and virulence of *C. sativus*.

Screening for resistance to *Phytophthora sojae*, *Fusarium graminearum* and *Pythium irregulare* in Nested Association Mapping (NAM) populations

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A Nested Association Mapping (NAM) population is currently in development which consists of 61 soybean genotypes that were all crossed to one common parent. Based on seed availability, 40 of the 61 lines were evaluated for resistance to three soil borne pathogens. Soybean genotypes were inoculated with six different *P. sojae* isolates using the hypocotyl assay to determine the presence of specific *Rps* genes. Partial resistance to *P. sojae* was assayed with a layer test, *F. graminearum* with a seed inoculation, and *Py. Irregulare* with sand-cornmeal based inoculum in a cup assay. Twenty-five lines (approximately 68%), including IA 3023, were susceptible to R1 of *P. sojae*, indicating they do not have any *Rps* genes. However, 26 lines had root score <5.0 indicating moderate to high levels of partial resistance. These and other results indicate that there may be a few populations within the NAM study that may be useful for identifying resistance loci for soil borne pathogens.

Evaluation of the efficacy of gaseous ozone treatment in eliminating *Clavibacter michiganensis* subsp. *michiganensis* on greenhouse surfaces

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Ozone, a strong broad-spectrum biocidal compound, has been used as a sanitizer in the greenhouse industry for many years. However, it is unknown whether gaseous ozone can effectively kill *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), the pathogen causing tomato bacterial canker, on greenhouse surface materials. In this study, the efficacy of gaseous ozone treatment in eliminating Cmm on four different types of greenhouse surface materials including concrete, trolley frame (iron with green paint), gutter (steel with white paint) and glass was evaluated. Three tests of ozone treatment efficacy against Cmm were set up in two greenhouses; greenhouse X (twice; 24 Acres, Maine, U.S.A.) and Y (once; 30 Acres, Ontario, Canada) with maximum concentrations of 0.4 ppm, 6.0 ppm, and 0.6 ppm, re-

spectively. The surface materials were inoculated with 10⁸ CFU of Cmm and placed in different locations in the greenhouse. There were no significant differences in Cmm populations recovered on the ozone treated surface materials compared with non-treated controls in the first test, but dramatic reductions (2–4 log) were found on trolley frame, gutter and glass surfaces in the second and third tests. There was no reduction in the Cmm population on concrete treated with ozone gas in the first and second tests and a reduction of only approx. one order of magnitude in the third test compared to the non-treated control. This pioneering study indicated that the efficacy of gaseous ozone treatment was greatly affected by ozone concentration and the texture of the greenhouse surface materials.

Novel sources of resistance to Maize rayado fino virus (MRFV)

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Maize rayado fino virus (MRFV) is one of the most important maize virus diseases of Mexico, Central and South America. Severe yield losses, ranging from 10 to 50% in maize landraces to nearly 100% in some modern cultivars, have been reported. The disease is insect transmitted, and is often found in a disease complex known as “achaparramiento” or red stunt. Maize genetic resources were evaluated in order to identify novel sources of resistance to *Maize rayado fino virus*. Beginning seven days post insect inoculation, disease incidence and severity were evaluated. Most of the lines were susceptible to MRFV, with average disease incidence ranging from 21 to 96% and severity ratings ranging from 1.0 (chlorotic spots on less than 25% of the plant) to 4.3 (severe striping on more than 75% of the plant). A few genotypes, including CML333 and Kil1, showed intermediate levels of resistance, with 14% and 10% incidence, respectively. Novel sources of resistance, with incidence of less than 5% and severity ratings of 0.4 or less, included inbred lines Oh1VI, CML287, and Cuba. The discovery of novel sources of resistance in inbred lines adapted to temperate and tropical regions will facilitate the identification of virus resistance genes and their incorporation into breeding programs.