2013 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in Manhattan, Kansas, June 12–14, 2013. 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: Abeyssekara, N. S., and Robertson, A. E. 2013. Analysis of genetic changes underlying pathotype shift in *Phytophthora sojae* isolates recovered from a soybean cultivar rotation study. (Abstr.) *Phytopathology* 103(Suppl. 3):S3.1. <http://dx.doi.org/10.1094/PHYTO-103-9-S3.1>

Analysis of genetic changes underlying pathotype shift in *Phytophthora sojae* isolates recovered from a soybean cultivar rotation study

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Phytopathology 103(Suppl. 3):S3.1

Phytophthora sojae is an oomycete pathogen causing the stem and root rot of soybeans. The pathotypes of *P. sojae* isolates are shown to rapidly change in the field as well as in culture. In a previous study, the effect of cultivar rotation on pathotype shifts in *P. sojae* was investigated. One hundred and twenty isolates were recovered over a four-year period. The study was initially inoculated with a single isolate, PS01 that was virulent only on soybean lines carrying *Rps7*. A subset of 22, 20, 15, and two isolates that were shown to gain virulence on *Rps1a*, *Rps1c*, *Rps1d*, and *Rps3b*, respectively were further analyzed. Previous research showed that virulence on *Rps1a* and *Rps1c* was gained either by deletion of the gene or the loss of transcript, while a deletion in *Avr1d* gene is known to result in virulence. An amino acid change or a frame shift may cause an avirulent strain to gain virulence on *Rps3b*. Polymorphisms in the *Avr* effector genes *Avr1a*, *Avr1c*, *Avr1d*, and *Avr3b* in the virulent isolates from the rotation study were investigated by amplifying the gene regions with PCR primers obtained by previously published research (except for *Avr1c*). PCR products of *Avr1a* and *3b* were amplified from the avirulent isolate, PS01, as well as all virulent isolates on *Rps1a* and *Rps3b*. Similarly *Avr1c* was amplified in PS01 but there was inconsistency in the presence and absence of the PCR fragment in some virulent strains. PCR amplification of *Avr1d* is in progress. Transcript and sequence polymorphisms, and copy number variations of this subset of isolates are currently being investigated. The results of this study will further strengthen the knowledge on the mechanisms by which *P. sojae* isolates change pathotype to gain virulence on existing *Rps* genes.

Genetic diversity of Barley yellow dwarf virus (BYDV) populations in Kansas wheat fields

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Phytopathology 103(Suppl. 3):S3.1

The Barley yellow dwarf complex is one of the most widespread and economically important assemblages of viruses infecting wheat. The genus *Luteovirus* includes at least three recognized virus species that are members of the disease complex: *Barley yellow dwarf virus* (BYDV)-PAV, -PAS, and -MAV. BYDV-PAV is the type species and historically, it has been the primary virus species found in Kansas. To characterize the genetic diversity of

BYDV-PAV and related BYDV species infecting wheat, a 2.3 kb genomic region was amplified and Sanger-sequenced from 57 and 75 single-plant isolates collected during the spring of 2011 and 2012, respectively. These samples were part of a statewide winter wheat virus survey. Phylogenetic analysis of the amplicon sequences using GenBank BYDV reference sequences revealed that the KS isolates belong to the PAV and PAS species, with predominance of PAS isolates (68.4% in 2011, 89.3% in 2012). Within the PAS species, the sequences clustered into two clades (designated herein as PAS-I and PAS-II). However, only 3% of the PAS isolates grouped into PAS-I. The nucleotide diversity (P_i) within clades was lower in PAS-II ($P_i = 0.006$) compared to PAS-I ($P_i = 0.019$) or PAV ($P_i = 0.017$) in both years. The results of this two-year study indicate enrichment and genetic stability of BYDV-PAS-II genotypes in KS wheat fields. Further investigations of the biological relevance of the occurrence and establishment of PAS-II in KS are underway.

Evaluation of global spring wheat germplasm for resistance to tan spot *Pyrenophora tritici-repentis* race 1

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Phytopathology 103(Suppl. 3):S3.1

Tan spot, caused by the ascomycetes fungus *Pyrenophora tritici-repentis* (Ptr), is a major foliar disease of wheat worldwide. Multiple races have been identified within the Ptr population and race 1 is the most prevalent race throughout the world's major wheat growing countries. Using resistant cultivars as a disease management strategy for tan spot is generally considered economical, durable, and environmentally friendly. In this study, 414 wheat genotypes from the CIMMYT elite wheat germplasm nurseries were screened for their reaction to Ptr race 1, by inoculating them individually with spores suspension at the two-leaf stage in the greenhouse. Of 414 genotypes tested, 69, 144, and 201 were rated as resistant, moderately susceptible, and susceptible to tan spot, respectively. The resistant genotypes with good agronomic characters can be used directly as tan spot resistant cultivars for tan spot in countries where they show adaptability or as sources of resistance in other wheat breeding programs. In an attempt to identify sources of resistance to multiple leaf diseases, genotypes that showed resistance to Ptr race 1 are presently under further investigation for their reaction to Ptr races 3, 5, and *Stagonospora nodorum* leaf blotch, *Septoria tritici* blotch, and spot blotch.

Evaluation of fungicides to effectively control the buffalograss false smut causal pathogen

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Phytopathology 103(Suppl. 3):S3.1

False smut caused by *Cercospora seminalis* is an important disease of turf-type buffalograss (*Buchloë dactyloides*) seed production. The disease prevents normal caryopsis development and causes considerable yield loss and reduced seed germination. Since there are no labeled fungicides for false smut, we

The abstracts are published as submitted. They were formatted but not edited at the APS headquarters office.

<http://dx.doi.org/10.1094/PHYTO-103-9-S3.1>

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studied in vitro sensitivity of *C. seminalis* to six commonly used turfgrass fungicides, namely thiophanate-methyl, boscalid, iprodione, propiconazole, azoxystrobin and pyraclostrobin. Growth inhibition of one fast growing and one slow growing isolate of the pathogen was monitored on petri plates amended with four concentrations (0.1, 1, 10 and 100 mg a.i./liter) of each commercial formulation of fungicide with three replications. The experiment was repeated and data were pooled for the analysis. Both isolates of *C. seminalis* were not sensitive to thiophanate-methyl. The other fungicides except for azoxystrobin had high activity against *C. seminalis* with EC50 varying from 0.02 to 0.17 mg a.i./liter. The azoxystrobin had a moderate EC50 value of 1.2 mg a.i./liter. A field study conducted to evaluate timing effect of application of azoxystrobin (i.e. before flowering, early flowering, late flowering and when disease symptoms are visible) did not give any significant results which were consistent with our in vitro results. Additional field tests for other fungicides are needed to determine if in vitro results are repeatable under field conditions.

Efficacy of disinfectants in killing urediniospores of wheat stem rust and soybean rust

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Phytopathology 103(Suppl. 3):S3.2

Current policy for disinfecting hard surfaces within the University of Minnesota Biosafety Level-3 (BSL-3) containment facility requires application of 70% alcohol followed by a 30 minute treatment interval. Because this treatment requirement is unsupported by empirical data and is laborious, cumbersome and time consuming, we conducted a series of trials to compare the efficacy of two common antimicrobial agents applied to kill urediniospores of wheat stem rust (*Puccinia graminis* f. sp. *tritici* race TTKSK) and soybean rust (*Phakopsora pachyrhiza*) when applied for much shorter time intervals. The two disinfectants, either a 70% alcohol solution or a 10% bleach solution (household sodium hypochlorite), were applied to viable urediniospores of the two pathogens for three time intervals: 2, 5 and 15 minutes. As an untreated control, deionized water was applied to viable urediniospores for the same three time intervals. The disinfection treatment was terminated by rinsing the urediniospores from the slide into a microtube with deionized water. The urediniospores were collected and assessed for viability, germ tube formation when placed on water agar. Both the 70% alcohol and 10% bleach solution treatments killed 100% of the urediniospores within 2 minutes and therefore would serve as effective disinfectant agents for these rusts. Because of its greater stability, ease of handling, and safety, 70% ethanol would be the preferred disinfectant agent for use in the BSL-3 facility.

Evaluation of *Triticum durum* germplasm collections for resistance to *Puccinia triticea*

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Phytopathology 103(Suppl. 3):S3.2

Resistance gene diversity to rust pathogens is necessary to ensure yield stability across *Triticum durum* production areas. Research efforts are underway to determine rust resistance gene diversity available in durum wheat germplasm and to identify underutilized and new sources of rust resistance that can be employed to expand the genetic diversity of durum wheat in breeding programs. The objective of this project is to identify effective sources of resistance that can be utilized to diminish yield losses by means of host resistance. Therefore, *T. durum* landraces were evaluated for their reaction to leaf rust race BBGQ. This race is similar in virulence to races previously identified and currently present in Mexico (BBG/BN) which have caused serious yield losses to Mexican farmers in the past decade. Despite BBGQ is not identified in the U.S.A. other than California, it could potentially move to the northern Great Plains, where the vast majority of US durum wheat is produced. A total of 497 landraces and cultivar accessions from the USDA/ARS-National Small Grain Collection (NSGC) were evaluated at seedling stage and molecular markers were used for the identification of the possible presence of previously known resistance genes to leaf rust in durum. The screening was done mainly for two resistance genes; Lr14a which is one of the best characterized sources of resistance in durum and Lr46 which confers slow rusting to leaf rust. Resistant genotypes were identified in low frequency of 5% and those resistant accessions were identified to be from 19 countries including: Algeria, Argentina, Australia, Cyprus, Ethiopia, Former Soviet Union, France, Iran, Italy, Malta, Nigeria, Poland, Portugal, Saudi Arabia, Tunisia, Turkey, the United States, China and Yemen. Marker assisted selection and development of biparental populations is being conducted to determine the genetic location of the resistance genes and their mode of inheritance.

Identification of *Frankliniella occidentalis* proteins that respond to or interact with Tomato spotted wilt virus

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Phytopathology 103(Suppl. 3):S3.2

Tomato spotted wilt virus (TSWV) is considered to be one of the ten most devastating plant viruses. In nature, TSWV is transmitted from plant to plant by thrips in a persistent propagative manner. The western flower thrips, *Frankliniella occidentalis*, is one of the most important vectors of TSWV. Little is known about the molecular aspects of TSWV entry and replication in tissues and organs of its insect vector. The overall goal of this work was to identify thrips proteins that directly interact with TSWV and those that respond to the virus infection of the insect vector. Using two-dimensional (2-D) gel electrophoresis and mass spectrometry, we identified 26 protein spots that contained 37 thrips proteins that displayed differential abundances in response to TSWV infection. Gene ontology assignments for 32 of these proteins revealed biological roles associated with the infection cycle of other plant- and animal-infecting viruses and antiviral defense responses. We have conducted virus overlay assays to identify thrips proteins that directly interact with TSWV. Our experiments revealed that four unique protein bands specifically interacted with purified TSWV virions. Identification of thrips proteins that interact with TSWV during infection provides new insights into the molecular basis of this interaction and generated a suite of candidate genes that can potentially be used as targets to deter thrips damage and tospovirus transmission.

A tolerance based index for effective screening of sorghum accessions against stalk rot diseases

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Phytopathology 103(Suppl. 3):S3.2

Stalk rots are major fungal diseases of sorghum [*Sorghum bicolor* (L) Moench] worldwide. They cause \$15 million annual economic loss in Kansas alone and there are no straightforward methods to control these diseases. Therefore, screening advanced breeding lines before release for stalk rot resistance is important for enhancing productivity. Conventionally, stem lesion length (LL) produced due to infection by fungal pathogens including *Fusarium thapsinum* (Fusarium stalk rot, FSR) and *Macrophomina phaseolina* (charcoal rot, CR) has been measured to evaluate the degree of plant resistance. Genotypes with lower lesion lengths are considered more resistant. In these genotypes is assumed that disease impact upon yield would be less under high pathogen pressure than in the susceptible. However, recent reports revealed contradictory findings concerning the correlation between yield and lesion length, which question the validity of using LL as a sole resistance indicator. The newly formulated *resistance-tolerance index* (IndexRT) was used to rank 36 advanced sorghum male sterility maintainers (B lines) in summer 2012. The IndexRT ranked ARCH 11035B, -11018B and -11030B lines as having the best performance against FSR, whereas the same lines were ranked at 1, 15 and 12, respectively, based upon mean LL alone. Similarly, ARCH 11018B, -11009B and -11006B performed best against CR, whereas the same lines were ranked at 2, 17 and 15, respectively, based upon their mean LL. There was no significant correlation between mean LL and the corresponding IndexRT ranks of lines, revealing the independence of the two ranking systems even though mean LL is an integral component of the IndexRT. The best performing B lines, selected using the IndexRT will be utilized for hybrid development. Steps involved in IndexRT formulation through incorporation of both resistance and tolerance, and its importance in critically enhancing sorghum productivity will be discussed.

Transformative technologies from knowledge of plant virus - aphid vector interaction

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Phytopathology 103(Suppl. 3):S3.2

Aphids transmit more than 275 plant viruses that result in considerable economic loss within the agricultural sector. Viruses in the Luteoviridae are obligately transmitted by aphids in a persistent manner that requires virion accumulation in the aphid hemocoel. To enter the hemocoel, the virion must bind and traverse the aphid gut epithelium. The molecular mechanisms involved in this process are poorly understood. By screening a phage display library, we identified a peptide that binds to the gut epithelium of the pea aphid *Acyrtosiphon pisum* (Harris) and impedes the uptake of Pea enation mosaic virus from the pea aphid gut into the hemocoel. In this talk, the development of two novel aphid management technologies based on knowledge of pea aphid - Pea enation mosaic virus molecular interactions

will be described. These technologies provide enhanced delivery of both gut active and neurotoxic peptides.

Virulence and diversity of Soybean cyst nematode field populations in Nebraska

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Phytopathology 103(Suppl. 3):S3.3

Soybean cyst nematode (SCN) is one of the most economically important soybean pathogens in the United States. Currently, the best management practices are the use of resistant cultivars and crop rotation. Though there are several genetic sources of SCN resistance, most of the SCN-resistant cultivars are derived from a single resistance source (PI 88788). In other states, an increase in virulence to PI 88788 has been reported due to prolonged use of this resistance. The objective of this study was to determine the virulence of SCN field populations and their diversity in Nebraska. SCN populations were randomly selected from soil submitted for diagnostic tests from SCN-infested fields across the state. Over 30 counties were represented in the 70 populations tested. After increasing SCN populations on a susceptible cultivar in the greenhouse, HG Type tests were performed. Over 40% of populations were virulent on PI 88788 with a maximum of 54% reproduction. Over 90% were virulent on PI 5484316 (Cloud) with a maximum of 63% reproduction. No populations were found to be virulent on PI 437654 (Hartwig). Virulence to PI 88788, 209332, and 5484316 were most common and found in nearly every county. Many counties also had populations that were virulent on PI 548402, 90763, and 89772. Information on virulence and diversity of SCN in Nebraska will provide insight for development and selection of SCN resistant cultivars.

A trend study of the confirmed incidence of Stewart's wilt of corn, *Pantoea stewartii* subsp. *stewartii*, in the U.S.A. (2001–2012)

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Phytopathology 103(Suppl. 3):S3.3

Pantoea stewartii subsp. *stewartii*, causal agent of Stewart's wilt of corn, is classified as a quarantine pest by more than 100 countries to which the US exports corn seed. A current review of the Widely Prevalent Bacteria of the United States, <http://www.prevalentbacteria.org>, states that this bacterial pathogen is found across the Midwestern Corn Belt states. A collaborative effort between public and private entities has resulted in a formal review of the confirmed reports of this disease from 2001–2012. Diagnostic laboratory confirmations of Stewart's wilt were compiled from data extracted from the NPDN (National Plant Diagnostic Network) National Repository records maintained by CERIS - Purdue University and the DuPont Pioneer Plant Diagnostic Database for the aforementioned time period. Stewart's wilt positive identifications in the DuPont Pioneer database indicate the disease was increasing in incidence each year from 2001–06, and the NPDN data show the same trend for the information available from 2004–06. It is believed that the lack of Stewart's wilt NPDN records from 2001–03 is a direct result of the records not being uploaded to the National Repository during this time period, rather than the disease not being observed or detected. Positive records of this disease from both databases totaled 222 in 2007. Both databases indicate a significant drop in Stewart's wilt positives in 2008 followed by very few confirmations in the past four years. Total number of U.S.A. Stewart's wilt confirmations from 2009–2012 were <20 based on these diagnostic records. These diagnostic reports are not a result of a concerted survey effort per se. They do include samples submitted from phytosanitary field inspections, where it is a requirement to submit a sample of suspected Stewart's wilt plants. This trend suggests that Stewart's wilt is not currently a prevalent bacterial disease in the corn growing areas of the United States.

Susceptibility of potato cultivars to *Potato Mop-top Virus*-induced tuber necrosis

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Phytopathology 103(Suppl. 3):S3.3

Fourteen potato cultivars representing three market classes were grown in 2011 and 2012 in a field in North Dakota known to be infested with *Spongospora subterranea* vectoring the *Potato Mop-top Virus* (PMTV). The objective of the experiment was to assess the susceptibility of commonly grown potato cultivars for the development of tuber necrosis caused by PMTV. Results of tuber necrosis assessments conducted over a seven month storage period demonstrated significant ($p < 0.0001$) differences in incidence and severity among cultivars. Red Norland and Red LaSoda were the most susceptible while Bannock Russet and Dakota Trailblazer were the least susceptible. The incidence of PMTV necrosis in cultivars ranged from zero to 40% while severity indices ranged from 0 (no necrosis) to 1 (presence of necrosis throughout the tuber). The incidence of PMTV necrosis increased

significantly during storage across most cultivars. Reverse transcription polymerase chain reaction (RT-PCR) was used to verify the presence of PMTV in representative samples. The results obtained in this study demonstrate that field trials can be used to effectively evaluate the susceptibility of potato cultivars to PMTV-induced tuber necrosis. These data will provide growers with useful disease management information to avoid highly susceptible cultivars and potentially replace them with less susceptible cultivars in the same market class. In addition, the data can be used for the development of PMTV resistant germplasm that can be utilized in future breeding strategies.

Seedling resistance to wheat leaf rust in Thatcher isolines carrying race specific and race non-specific genes

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Phytopathology 103(Suppl. 3):S3.3

Leaf rust caused by *Puccinia triticina* is the most prevalent disease of wheat worldwide, causing significant yield losses. To date 67 leaf rust resistance (*Lr*) genes have been designated but only a few are effective against the highly diverse North American *P. triticina* population. The use of broad, non-race specific resistance has been proposed as an alternative to achieve durable rust resistance. Post-haustorial resistance is generally involved in race specific resistance and is characterized by hypersensitive reaction (HR). Pre-haustorial resistance does not generally involve an HR, and is presumed to be more durable and may be involved in broad and non-race specific resistance. In this study Thatcher near isogenic lines (NIL'S) carrying different *Lr* genes Thatcher *Lr*9, Thatcher *Lr*21, Thatcher *Lr*34, Thatcher *Lr*35 and barley [near non-host] cultivars Q21861 and Harrington were characterized for pre- and post-haustorial resistance components using races THBL, MCDL inoculation in time course experiments at seedling plant stage. Pathogen infection structure development, colony size plant and tissue responses were evaluated using fluorescence microscopy, and disease infection type scored at 14 days post inoculation were compared to the microscopic observations. When challenged with two races, NILs Tc-*Lr*2a, -*Lr*2c and -*Lr*34 and barley cv. Harrington and Q21861 differed in number and time of fungal structure development compared to susceptible cv. Thatcher. Implications of research findings in dissection of host-pathogen interactions and future applications of this technique in breeding for durable rust resistance will be discussed.

A spike-based simulation model for wheat blast incidence

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Phytopathology 103(Suppl. 3):S3.3

Wheat blast caused by *Magnaporthe oryzae* was first reported in 1985, in the state of Paraná. The disease rapidly spread into other sub-tropical wheat-growing regions of South America, becoming a primary concern in those regions. Warm temperatures and prolonged wetness during wheat growth and development stages favors disease development. The pathogen infects all aboveground parts of the wheat plant, but infection of the wheat heads lead to higher yield losses that may vary from 10 to 100%. Disease control is limited by the lack of effective fungicides and resistant varieties. The pathogen might have evolved from *M. oryzae* isolates from other plant species, but its origin remains unknown. The sporadic nature of outbreaks is related to requirements of specific weather conditions for the disease to occur. Disease prediction models can be useful to evaluate the risk of a new disease to become established in a region. However, there is a lack of significant amount of quantitative data to build and test the models. Nevertheless, a disease cycle model that uses hourly temperature, rainfall and relative humidity has been developed and its outputs were compared with epidemic and non-epidemic years. The model correctly classified years in the state of Paraná, Brazil from 2000 to 2011. Recently, Bayesian modeling is being explored to improve predictions. The performance of the improved models will be presented and discussed.

Response of near isogenic sorghum lines, differing at the *P* locus for plant color, to grain mold and head smut fungi

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Phytopathology 103(Suppl. 3):S3.3

Leaves and stalks of many sorghum plants accumulate dark red or purple pigments upon wounding, while "tan" plants do not. Unpigmented "white" grain grown on tan plants is more desirable for food. The hypothesis tested was that these plant pigments protect grain from the panicle diseases grain mold and head smut. Near isogenic tan and purple plant color genotypes with

white grain were planted at Lincoln and Ithaca, NE, and Corpus Christi, TX. Field grown grain was plated onto semiselective media to detect the presence of sorghum grain-molding genera *Alternaria*, *Fusarium* and *Curvularia*. Results indicated that more *Fusarium* and *Curvularia* spp. were recovered from grain grown at Corpus Christi than at Nebraska; however there was no indication that grain from purple plants was more resistant to the three fungal genera than grain from tan plants. Most fungi identified morphologically were *Alternaria alternata*. Molecular identification of *Fusarium* species using translation elongation factor 1- α gene sequences, showed that *F. thapsinum* and *F. proliferatum* infected grain at all three locations. At Corpus Christi, head smut disease incidence was assessed, which is caused by the fungus *Sporisorium reilianum*. Surprisingly, purple plants had significantly greater disease incidence than tan plants. We propose that the tan plant color lines, which are promising for development of food grade sorghums, are not more susceptible than pigmented lines to either grain mold or head smut.

The response of soil microbial communities to organic and conventional fertilization: Comparing soil DNA and RNA analyses

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A central idea in organic agriculture is that organic management supports microbial communities that provide important ecosystem services. Phytopathology 103(Suppl. 3):S3.4

These services may include biocontrol by microbes and microbial contributions to nutrient cycling, soil organic matter formation, and plant nutrient uptake. We evaluated total and active microbial community responses in a field experiment comparing organic vs. conventional nutrient management for tomato production. The fertility treatment included low and high levels of organic or conventional fertility. Low fertility treatments in both organic and conventional management received nutrients only through a cover crop (buckwheat). The conventional high fertility treatment was potassium nitrate, calcium nitrate, and inorganic pre-plant fertilizer, and the organic high fertility treatment was fish hydrolysate and compost pre-plant fertilizer. Using 454-pyrosequencing, we compared total resident fungal, bacterial, and archaeal communities recovered from a DNA approach, and the actively metabolizing microbial communities recovered through RNA (cDNA). Our data suggest that organic management with high fertility may increase bacterial diversity compared to the other treatments. We recovered a number of bacterial genera with important agroecological roles, including *Nitrospira* and *Rhizobium*. Comparing cDNA and DNA, we found for example that there are differences in the frequencies of some fungal genera such as *Aspergillus*, *Glomus* and *Alternaria*. These differences between cDNA and DNA suggest different views of the microbial community that may provide insights about agroecological functions.

Predicting stripe rust epidemics using general estimation equations

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Phytopathology 103(Suppl. 3):S3.4

The objective of this research was to identify environmental conditions that are conducive for stripe rust epidemics in the central Great Plains region of the United States and to develop predictive models for epidemics in Kansas. Predictive models were developed based on historical estimates of disease related yield loss and monthly summaries of meteorological variables and moisture indices that are likely associated with overwintering, infection, or reproduction of the rust pathogen. Boosted regression trees were used to determine the relative influences of weather-based predictor variables on stripe rust epidemics. The identified variables were evaluated in predictive models using general estimation equations (GEE). GEE's are useful for modeling clustered data by adding a second order variance structure to model the population average rather than using maximum likelihood derivations that can result in inflated standard errors estimates. The model error was estimated using a k-fold validation by year. Results indicate that stripe rust epidemics are strongly influenced by moisture conditions in the southern Great Plains during the fall and winter months. In the spring, temperature variables within Kansas appear to influence the intensity of stripe rust epidemics and may help refine estimates of local yield loss. These predictive models would help producers evaluate the risk of disease and decide if fungicides are needed to suppress disease related yield losses.

Detection of seed to seedling transmission of *Colletotrichum lindemuthianum* in dry edible pinto beans

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Phytopathology 103(Suppl. 3):S3.4

Anthrachnose of dry bean (*Phaseolus vulgaris* L.), caused by the fungal pathogen *Colletotrichum lindemuthianum* Sacc. & Magnus, can cause seed

color distortion, large reductions in yield, and reduced seed quality. Although widespread losses have not been observed in North Dakota, the lack of host resistance and favorable environmental conditions could lead to substantial economic losses due to this disease. The introduction of *C. lindemuthianum* into a field most commonly occurs through planting infected seed, therefore, the most important management practice is the use of certified seed. Inoculum for in-season infections can be produced from seed to seedling transmission and on crop residue, resulting in rain-splashed or wind dispersed spores. Early infections caused by seed to seedling transmission of *C. lindemuthianum* can be devastating and have been proven to be the most important factor in disease development. Seed to seedling transmission has been documented to be as high as 15% in symptomless seed. Previous research has demonstrated an increase in seedling disease with increasing symptom severity on seed; however, pathogen presence was not evaluated, and seed to seedling transmission has not been studied under North Dakota growing conditions or using local cultivars. The detection of *C. lindemuthianum* in dry bean seed traditionally has been accomplished using visual evaluation for pathogen. These methods are labor intensive and may not be sensitive enough to detect symptomless seed infections. A real-time PCR assay was developed recently for quantifying *C. lindemuthianum* in seed using primers developed from the ITS region. The purpose of this study is to evaluate the utility of real-time PCR assays to aide in the evaluation of seed to seedling transmission and for the detection of symptomless seed infections.

Networks of stored wheat diseases and pests: A framework to create new management strategies

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Phytopathology 103(Suppl. 3):S3.4

Stored wheat is exposed to a dynamic process from harvest to its final destination in the milling industry. Pathogens and pests of stored wheat move through complex dynamic networks linking fields, farms, grain bins, grain elevators, and other bulk storage facilities. Human transport and other forms of pest dispersal link the components of this network. A new strategy for creating novel sampling and mitigation strategies, and for improving implementation of the current strategies, will be the use of network models for pathogen and pest movement through stored grain systems using information about the network structure. An understanding of network structure can be applied to identifying the key network components for pathogen or pest movement through the system. Identification of a crucial network node, such as terminal elevator, will be particularly important for implementation of sampling, management, and mitigation strategies. The importance of these nodes will be driven by the grain movement from a large number of fields and small storage facilities to larger facilities. In some cases more detailed information about network structure can identify key nodes that link two large sections of the network, such that management at the key nodes will greatly reduce the risk of spread between the two sections. In addition to the spread of particular species of pathogens and pests, we also evaluate the spread of problematic subpopulations, such as pesticide resistant groups of individuals or the introduction of new pests with uncommon traits. We present a conceptual framework for the analysis of stored wheat pathogen and pest networks for Australia and the United States.

Genetic structure and effective population size of *Fusarium verticillioides* (Saccardo) Nirenberg populations from two maize (*Zea mays* L.) hybrids grown at 12 locations and 8 states in the U.S.

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Phytopathology 103(Suppl. 3):S3.4

Fusarium verticillioides is the most common causal organism of stalk and ear rot of maize worldwide and also produces fumonisin mycotoxins. We analyzed the genetic structure of *F. verticillioides* collected from two maize hybrids (P3377, P3475) grown at 12 locations in 8 states from the Midwest and Great Plains regions of the U.S. We tested the hypothesis that individuals within those 12 populations were genetically diverse due to random mating and that populations from different locations and the two maize hybrids were genetically distinguishable from one another. Two amplified fragment length polymorphism (AFLP) primer pairs were used to resolve 66 polymorphic loci from 322 isolates. Population structure analysis with $\geq 5\%$ allele frequency loci (24 loci) supported K=3 genetic clusters which presented 63%, 56% and 62% genotypic diversity. There was evidence of linkage disequilibrium

($P < 0.01$) indicating that sexual reproduction was not frequent. Gene flow between fungal population from the two maize hybrids was high ($N_m = 7.38$) suggesting that these two population were not isolated from one another. Potential for drift was highest in Algona (IA), Marion (IA) and North Platte (NE) as evidenced by the high percentage of female sterile and associated reduction in inbreeding effective populations number $N_e(f)$. Periodic monitoring of this pathogen population is needed to identify new genotypes and/or groups present in maize growing areas of the United States. This study is the first report of significant genetic subdivisions within populations of *F. verticillioides* from maize.

Investigating the performance of reduced risk fungicides for control of carrot foliar diseases

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Phytopathology 103(Suppl. 3):S3.5

Alternaria leaf blight, caused by the fungus *Alternaria dauci*, and *Cercospora* leaf spot, caused by the fungus *Cercospora carotae*, are the most prevalent foliar diseases of carrot worldwide. These foliar pathogens reduce yield by limiting photosynthetic capacity and weakening the petioles needed for mechanical harvest. Several reduced-risk (RR) fungicides are available for the control of carrot foliar disease, however, the efficacy of these fungicides relative to fungicides currently used to control carrot diseases is not known. In 2011 and 2012, the timing and performance of the new RR fungicides for control of carrot foliar disease were evaluated. In both years, fungicide applications were initiated at the first sign of foliar disease and subsequent applications followed every two weeks. Foliar disease severity was measured using the Horsfall-Barrett scale and disease ratings were used to calculate an Area Under the Disease Progress Curve (AUDPC) for each treatment. Carrots were harvested at the end of the growing season to determine yield. In 2011, six fungicide treatments were tested against an untreated control (UTC). Carrots that were treated with Bravo Weather Stik, Quadris Top, Inspire XT, Omega 4C, and A16976 had significantly less foliar disease than the UTC. However, carrot yield was not different between the UTC and the fungicide treatments. In 2012, several additional fungicide treatments with a reduced number of applications and alternative application timings were included in the experiment. Although low disease pressure was observed in 2012, disease ratings in the UTC were significantly greater than all but two treatments. Four RR treatments resulted higher yields than the UTC. All of the highest yielding treatments included a fungicide application in each of the 5 bi-weekly applications. Results indicate that IPM programs for the control of carrot foliar diseases could be further refined to include new RR fungicides.

Screening of winter wheat germplasm for resistance to leaf and stripe rust

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Phytopathology 103(Suppl. 3):S3.5

Wheat leaf rust, caused by *Puccinia triticina*, and wheat stripe rust caused by *P. striiformis* f. sp. *tritici* (Pst) are important foliar diseases of wheat (*Triticum aestivum* L.) in the United States. Growing resistant cultivars is the most economical and environmentally friendly strategy of managing wheat rusts. Wheat leaf rust and stripe rust continue to threaten wheat production due to the constant emergence of virulent races of the pathogens. In this study, we evaluated winter wheat cultivars grown in North Dakota (WWR) and winter wheat genotypes from the NDSU winter wheat breeding program (WWNDSU) for seedling resistance to single pustule isolates of two *P. triticina* races, MCDL and TBDJQ, and one isolate of Pst, race PSTv-37, that are predominant in this region. The Pst isolate belongs to the newer populations of Pst races, identified in the Great Plains since 2000, that are more aggressive and better adapted to warmer temperatures. Thirty-nine (61%) of the WWR were resistant to race TBDJQ while 43 (74%) were resistant to the MCDL race. Only four cultivars (8%) were resistant to the PSTv-37 race. Three cultivars were resistant to both races of *P. triticina* and the Pst race. Forty-nine (43%) of WWNDSU were resistant to race TBDJQ and 52 (66%) showed resistance to race MCDL. However, only a few were resistant to the Pst race. This greenhouse experiment demonstrated that the majority of cultivars and genotypes were moderately resistant to both races of *P. triticina* and only a few were resistant to the Pst race. These results are useful in guiding rust resistance breeding efforts for our region as well as providing information to winter wheat growers so they can incorporate rust resistance information into their decision on which cultivars to grow.

Spurious symptoms caused by inoculum carriers used in plant disease resistance evaluation

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Phytopathology 103(Suppl. 3):S3.5

Artificially induced infection is commonly used for screening plant varieties for disease resistance. A standard technique for infecting soybean (*Glycine max*) with root rot pathogens consists of inoculation with infested seed as a substrate supporting the pathogen. In soybean variety screening trials uninfecting substrates appeared to affect shoot and root growth and caused lesion development like that caused by root rot pathogens. The objective of our research was to determine if uninfecting inoculum substrate affects soybean shoot or root growth and root symptom development. In an initial experiment uninfecting substrates, including soybean, lentils, oats, popcorn, red and white sorghum, navy beans, and sudangrass, reduced plant height as much as 30% when compared to infested substrates. When soybeans were planted in media mixed with a 20:1 ratio of soil to seed of red sorghum, white sorghum, or sudangrass that was either uninfecting or infested with *Fusarium solani* the uninfecting seed caused root lesion development and shoot biomass and root biomass were reduced by 13% and 18% respectively when compared with the effect of infested seed. Inoculation with uninfecting red sorghum reduced soybean root weight by more than 34% compared to respectively either a 6% or a 13% reduction occurring when white sorghum or sudangrass seed was added. In a subsequent experiment the reduction in both plant height and weight increased with increasing concentrations of the substrate. Uninfecting inoculum substrate caused both reduced growth and spurious symptom development that may mislead the researcher conducting soybean variety screening for disease resistance.

Genetic and virulence variation of *Magnaporthe oryzae* from wheat and weed Poaceae

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Phytopathology 103(Suppl. 3):S3.5

Blast, caused by *Magnaporthe oryzae*, produces considerable damage on the wheat crops in Brazil. The emergence of this pathogen is relatively recent, dating from the middle of the last eighties, when the first outbreaks of the disease were detected in Paraná State. Wheat genotypes, apparently resistant, demonstrate susceptibility in other regions of the country, indicating interaction between genotype and environment besides strong influence of genetic-phenotypic variability in the pathosystem. Moreover, previous studies indicated very low genetic migration between wheat and rice populations of *M. oryzae*. This statement suggests that the Brazilian population of *M. oryzae* adapted to wheat was probably derived from an unknown population of *M. oryzae* infecting other grasses, and not from rice *M. oryzae* populations. Now it is being developed a work in order to verify the level of genetic complexity and virulence of *M. oryzae* populations from wheat and wheat weed Poaceae in Brazil. Therefore, these two types of isolates obtained in different Brazilian wheat regions are being classified according to molecular and virulence criteria using, respectively, microsatellite markers and inoculation procedures on a set of wheat genotypes with differential reaction to the disease. One of the expectations of the work is to verify the actual role that weed Poaceae represent in the development of blast in wheat crops in Brazil, participating directly in the generation of variability of the fungus, as substrate survival of the fungus and/or source of inoculum for the disease.

Efficacy of metalaxyl and ethaboxam on reducing corn seedling blight in naturally infested soil at two temperatures

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Phytopathology 103(Suppl. 3):S3.5

Farmers are planting corn earlier in the growing season to maximize yields. This increases the risk of corn seedling blight due to the likelihood of planting in cool, wet soils, which favor *Pythium* species. Farmers use fungicide seed treatments to manage corn seedling blight; however, little is known about the impact of temperature on the efficacy of various fungicides to minimize seedling diseases. The objective of this study was to determine the efficacy of two fungicides on reducing corn seedling blight at two temperatures. Corn seed treated with metalaxyl (5 gai/100 kg of seed), ethaboxam (1 gai/100 kg of seed), or metalaxyl and ethaboxam (5 and 1 gai/100 kg of seed, respectively) was planted in naturally infested soil obtained from four locations in southeast Iowa known to have corn seedling disease caused by *Pythium* spp. Fungicide efficacy was evaluated at 25C and 13C by assessing seedling emergence (rating scale 0 or 1), and mesocotyl and root rot severity (rating scale 0–5) per seedling. At 25C and 13C, emergence ratings were greater ($P < 0.05$) for each fungicide treatment compared to the control. Mesocotyl and root rot severity ratings differed ($P < 0.05$) between fungicide treatment and the control for both temperatures. Root rot severity ratings were consistently greater at 13C than 25C across all fungicide treatments. At each temperature, the combination of metalaxyl and ethaboxam increased emer-

gence ($P < 0.05$) and decreased mesocotyl and root rot severity, compared to metalaxyl or ethaboxam alone ($P < 0.05$). *Pythium sylvaticum* and *P. lutarium* were recovered from corn seedlings grown at 25F and *P. torulosum* was isolated from corn seedlings grown at 13C.

Fungicide sensitivity of *Pythium* species affecting corn and soybean in Iowa at three temperatures

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Phytopathology 103(Suppl. 3):S3.6

Corn and soybean farmers in Iowa are planting earlier in the growing season when soils are cool and wet, thereby increasing the risk of seedling disease development. As a result, farmers use fungicide seed treatments to help manage seedling disease. The effect of temperature on fungicide sensitivity of *Pythium* spp. has not been studied, and since this may affect efficacy of a seed treatment, we evaluated fungicide sensitivity of the most prevalent *Pythium* spp. affecting corn and soybean in Iowa at three temperatures in vitro. *Pythium torulosum*, *P. oopapillum*, *P. lutarium*, and *P. sylvaticum* were evaluated for fungicide sensitivity at 23C, 18C, and 13C using a plate assay. All *Pythium* spp. were evaluated for fungicide sensitivity to metalaxyl, ethaboxam, azoxystrobin and pyraclostrobin. *Pythium torulosum* was sensitive to ethaboxam (>50% mycelia inhibition), but insensitive (<50% mycelia inhibition) to metalaxyl at 23C. *Pythium sylvaticum* was sensitive to both metalaxyl and ethaboxam at 23C, but was insensitive to azoxystrobin and pyraclostrobin at 23C; however, it was sensitive to the strobilurins at 13C. *Pythium oopapillum* and *P. lutarium* were both insensitive to pyraclostrobin at all temperatures. All four species of *Pythium* are currently being screened at the above three temperatures for fungicide sensitivity on corn and soybean in a greenhouse assay.

Greenhouse corn hybrid responses to infection by *Clavibacter michiganensis* subsp. *nebraskensis*

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Phytopathology 103(Suppl. 3):S3.6

Clavibacter michiganensis subsp. *nebraskensis* (Cmn) is a Gram positive bacterium that causes Goss' wilt and leaf blight on corn. Yield loss is dependent on the percent leaf area blighted during the grain fill period. To improve our understanding of the mechanism of resistance of corn to infection by Cmn, leaves of a susceptible and a tolerant hybrid at the V4 growth stage were wound-inoculated. Leaf blight progress was monitored over time as lesion length along the leaf blade, while movement of Cmn from the point of inoculation was enumerated using dilution plating. Lesions on the susceptible hybrid were more than 60 cm in length compared to 40 cm on the tolerant hybrid ($P = 0.05$) at 23 days post inoculation (DPI). Progress of lesions towards the proximal end of leaves was less than 9 cm in the tolerant and more than 18 cm in the susceptible hybrid. Bacteria colonization of healthy tissues preceded lesion formation in both hybrids but was faster in the susceptible hybrid. Multiplication of Cmn in susceptible hybrid was ten-fold higher than in the tolerant hybrid leading to rapid death of colonized tissues. Tolerance to Cmn infection occurred approximately 14 DPI and a possible mechanism may be linked to the production of a dense matrix that restricts movement of bacteria cells. The nature of this matrix is currently being investigated. Understanding the mechanisms of resistance of corn to Cmn is key as the use of resistant hybrids underlies management.

Mobile technologies to increase efficiency and effectiveness

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Phytopathology 103(Suppl. 3):S3.6

Knowledge transfer within traditional Extension and educational programs is at a crossroads. The definition of "community" has evolved from interactions dominated by face-to-face instruction and traditional mass media to more personalized social networking websites and push-delivery technologies. Connectivity through mobile devices has played a pivotal role in this transition, with user adoption rates surpassing those set by early internet users. Given the movement toward wireless information delivery and limitations in resources, we must transform our methods of delivering information and interacting with stakeholders to maintain relevancy as well as confront grand challenges such as food security. For example, stakeholder feedback from an Area-wide Pest Management Program (AWPM) program revealed that a unified integrated pest management (IPM) program was needed for hard-red winter wheat producers in the Southern Great Plains. As a result, web-based applications (iwheat.org and my.iwheat.org) for use on mobile devices were developed to increase adoption of IPM tools in this AWPM program. The iWheat project in general is a multi-state effort that works to facilitate a long-term, area-wide management program for winter wheat. These applications are based on farm level processes, while allowing for field specific

management of key insect pests, weeds, and pathogens. Traditionally, Extension programs have relied on agent/educator centric, independent approaches to address producers' pest problems. Consequently, iWheat is one of the first unified, public-accessible pest-monitoring networks for wheat pest management currently in existence. Recent advances in mobile technologies and modes by which we access data have paved the way for more efficient, real-time approaches to connect pest monitoring and management information with pest management practitioners over wide geographic areas. Without easily accessible information and IPM recommendations, agricultural producers will fall back on non-sustainable, reduced-risk approaches that include prophylactic use of pesticides.

Screening the University of Illinois maize inbred collection for resistance to Goss's wilt

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Phytopathology 103(Suppl. 3):S3.6

Goss's wilt of maize, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), is a re-emerging disease in Illinois. The re-emergence of this disease in Illinois and the Midwestern U.S. has been due to an increase in inocula from growing continuous maize with limited rotation with non-host crops, a decrease in tillage that leaves inocula on the soil surface, and the production of susceptible maize hybrids. The University of Illinois maize inbred collection consists of over 2,000 inbred lines that have been collected and maintained for several years. An initial screen of this collection was conducted in a research field near Champaign, IL in 2011, in which the lines were inoculated with a Cmn cell suspension and rated for Goss's wilt severity using a 0–9 severity scale. From this initial screen, over 150 lines were identified that had moderate to high levels of resistance to Goss's wilt. These putative resistant lines were evaluated in a replicated, inoculated field trial near Champaign, IL in 2012. The mean Goss's wilt severity rating of this trial was 2.9, and fifteen of the lines had a severity rating of a 2.0 or less. The most resistant lines identified from this research are potential sources of resistance to Goss's wilt, and could be used in maize breeding programs to develop Goss's wilt resistant hybrids.

Incubation and latent periods of basil downy mildew (*Peronospora belbahrii*) as affected by temperature and wetness duration

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Phytopathology 103(Suppl. 3):S3.6

Effects of temperature (15, 20, 25 and 30°C) and post-inoculation wetness duration (0, 6, 12, 24, 48 or 96 h) on incubation and latent periods of basil downy mildew (BDM) were studied in growth chambers, air-conditioned (AC) and Fan & Pad (FAP) equipped greenhouses at the Tropical Research and Education Center in Homestead, Florida. Seeds of the sweet basil (*Ocimum basilicum*) 'Genovese' were grown in 10-cm pots for 3 weeks and seedlings were inoculated with *P. belbahrii* at 2×10^3 sporangia/ml. A linear interpolation method was used to determine incubation (IP) and latent period (LP). The time at which 50% of inoculated leaves showed symptom of the disease or sporulation was considered as IP or LP, respectively. In the AC and FAP greenhouses, IP and LP ranged between 6.3 to 8.3 days and 10.0 to 16.4 days, respectively. The average daily temperature was 25°C and relative humidity was 80% in these two greenhouses when the inoculation experiments were run from April to May, 2011. Ambient temperature and relative humidity in these greenhouses fluctuated between 21 to 32°C and 48 to 98%, respectively, depending on the time of the day. In growth chambers, BDM severity was significantly higher at 20 and 25°C compared to 15 and 30°C. At the same time, there was a significant increase in disease severity as wetness duration increased from 0 to 24 h. Further extended wetness duration did not cause any significant change in disease development. Results from this study indicate that proper manipulation of the environment and choice of planting time is vital for management of downy mildew on basil.

Efficacy of copper hydroxide in inhibiting the growth of new clonal lineages of *Phytophthora infestans*

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Phytopathology 103(Suppl. 3):S3.6

Phytophthora infestans causes late blight, one of the most important potato and tomato diseases worldwide. Repeated applications of fungicides are the primary method of disease control in season. For organic production, fungicide formulations containing copper are generally most effective, and while some new organic fungicides have been developed, little efficacy data is available. In whole plant trials under laboratory conditions with the US-23 lineage, the EF400 fungicide treatment showed significantly less disease than

the inoculated control, but, surprisingly, the copper hydroxide (Champ Formula 2) treatment showed disease not significantly different than the inoculated control. To further investigate this, colony diameter of one representative isolate from each of three recently emerged clonal lineages of *P. infestans*, US-22, US-23, and US-24, was measured on Rye A media amended with a range of copper hydroxide concentrations (100 ppm, 10 ppm, 1 ppm, 0.1 ppm, 0 ppm). For US-22, 100 ppm and 10 ppm copper hydroxide almost completely inhibited growth (93.8%, 91.7%, respectively), but 0% inhibition was seen at 1 ppm and 0.1 ppm. US-23 showed a similar trend with the 100 ppm and 10 ppm treatments demonstrating 100% inhibition compared to 21.7% and 1.31% for 1 ppm and 0.1 ppm, respectively. The US-24 clonal lineage appeared insensitive to copper hydroxide even at 100 ppm, showing only 20.9% inhibition when colony diameter was considered. Our data indicated that the effectiveness of copper hydroxide is dependent upon concentration or rate and that appropriate rates must be applied to achieve effective control of late blight caused by new clonal lineages of *P. infestans*.

Inhibition of vector transmission of Tomato spotted wilt virus (TSWV) by transgenic tomato plants that express the TSWV glycoprotein, G_N

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Phytopathology 103(Suppl. 3):S3.7

Tomato spotted wilt virus (TSWV) causes disease to many plants that are of economic importance worldwide, and the virus is vectored by thrips species, including *Frankliniella occidentalis*, the western flower thrips. We demonstrated previously that a soluble form of the TSWV G_N protein (G_N-S) binds to thrips midguts and inhibits virus acquisition and subsequent transmission. We generated transgenic tomato plants expressing G_N-S with a GFP tag (G_N-S::GFP). We hypothesized that G_N-S expressed in the tomato plants would reduce virus titer in the vector and transmission efficiency. To test this hypothesis, we inoculated transgenic tomato plants with TSWV and performed acquisition and transmission assays. The transgenic and non-transgenic tomato plants supported similar TSWV titers when measured by DAS-ELISA. Thrips fed virus-infected transgenic G_N-S::GFP tomato plants had significantly lower virus titers and transmission efficiencies than thrips fed on TSWV-infected non-transgenic tomato plants. These results demonstrate that an initial reduction in virus infection of the insect midgut can result in a significant decrease in virus titer and transmission over the life-span of the vector. The transgenic G_N-S::GFP plants are a promising new tool for basic studies of the G_N-thrips interaction and field experiments examining the utility of the transgenic plants in preventing secondary spread of TSWV.

Polyethylene Glycol (PEG) mediated transformation of *Septoria musiva*

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Phytopathology 103(Suppl. 3):S3.7

Septoria musiva is a fungal pathogen causing leaf spot and stem canker diseases of hybrid poplar. These diseases limit poplar production in many regions of North America. The mechanism of the host-parasite interaction between *S. musiva* and *Populus* spp. is poorly understood. As a result, an association mapping study will be conducted using a population of 200 isolates of *S. musiva* to identify putative virulence factors. The validation of these virulence factors will require the genetic manipulation of the pathogen. To achieve this, a simple and efficient transformation of *S. musiva* protoplasts, adapted from protocols for *Stagonospora nodorum* transformation, has been developed. Protoplasts of *S. musiva* were isolated using lysing enzymes and driselase. The protoplasts were transformed with a construct containing green fluorescent protein (GFP) and the hygromycin resistance gene as a selectable marker. The transformation was mediated using polyethylene glycol (PEG). The confirmation of transformants and the transformation efficiency of this method will be presented. The development of this protocol provides a tool to transform *S. musiva* protoplasts that will facilitate studies examining the infection biology of the pathogen, validation of candidate genes, and functional analysis of those genes.

Evaluation of greenhouse inoculation methods to screen germplasm for resistance to *Fusarium* root rot pathogens

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Phytopathology 103(Suppl. 3):S3.7

Dry field pea (*Pisum sativum*) production has increased substantially in the major production areas in the US, including North Dakota and Montana. Historically, *Fusarium solani* f. sp. *pisi* was regarded as the most common pathogen causing root rot of peas in the US. Within the past decade, *F. avenaceum* has been determined to be the major pathogen in the pea root rot

complex in North Dakota, and has been identified in other major pea production areas in the US and Canada. Very little information is available regarding sources of resistance to *F. avenaceum*. Research results from screening 21 commercial dry pea cultivars indicated that only one cultivar had some level of resistance to root rot caused by both pathogens. Also, recent research comparing disease severity of these two pathogens indicates that, while *F. solani* inoculated field trials had higher disease severity, those inoculated with *F. avenaceum* displayed lower emergence. This may indicate that, while both pathogens are causing root rots, *F. avenaceum* also may cause a higher incidence of seed decay or damping off. The management of this complex will necessitate a greater understanding of the etiology of each of these pathogens individually, and when both are present in the same environment. There will be an evaluation of three inoculation methods; the inoculum layer method, spore suspension method, and seed soak method. Viewing each species individually across a varied host population in combination of the two fungi across those genotypes displaying some resistance to either, or both species, will provide researchers and breeders with the tools to accelerate the development of root rot resistant cultivars.

Non-native soybean phytoalexins: Potential use in controlling soybean pathogens

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Phytopathology 103(Suppl. 3):S3.7

Production of secondary metabolites such as phytoalexins plays a pivotal role in innate plant defense. Phytoalexins are produced in response to infection and can be toxic to pathogens. However, naturally produced soybean phytoalexins do not provide sufficient defense to many economically important soybean diseases. Non-native phytoalexins could have the potential to increase soybean defense. Two non-native phytoalexins, resveratrol and pterostilbene, were evaluated *in vitro* in solid and liquid media for their ability to suppress growth of three economically important soybean pathogens, *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. For the solid medium test, each fungus was grown on potato dextrose agar (PDA) amended with 100 µg resveratrol, 200 µg resveratrol, 25 µg pterostilbene, 50 µg pterostilbene, or non-amended control. For the liquid medium test, 25 µg pterostilbene or 100 µg resveratrol were added to 50 ml potato dextrose broth (PDB) and compared with a control. When grown on PDA or PDB, pterostilbene and resveratrol significantly suppressed growth of all three fungal pathogens compared to the control. On PDA, pterostilbene averaged over both concentrations reduced growth by 60% compared to an averaged 13% reduction caused by resveratrol. In PDB, pterostilbene reduced growth by 46% compared to a 38% reduction caused by resveratrol. These results indicated that pterostilbene and resveratrol have significant fungal suppressing qualities and that transformation of soybean to produce these phytoalexins in addition to naturally occurring soybean phytoalexins could potentially increase innate resistance. In ongoing work, soybean hairy root cultures transformed to express the two non-native phytoalexins will be tested for fungal colonization after inoculation with *M. phaseolina* and *R. solani*.

Principal component analysis of soil texture and soybean cyst nematode populations before and after corn rotation in Nebraska

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Phytopathology 103(Suppl. 3):S3.7

Soybean cyst nematode (SCN, *Heterodera glycines*) remains the most yield-limiting pathogen of soybean in the North Central Region of the U.S. The use of resistant soybean varieties and crop rotation are two effective practices to manage the disease. In this study, SCN population change after corn rotation was assessed in 45 commercial fields in Nebraska, and principal component (PC) analysis was used to investigate the relationships of soil texture, and SCN population densities (eggs/cm³ of soil) before (P_i) and after the rotation year (P_f). The study included 21 fields in 2009, 18 fields in 2010 and six fields in 2011. Both P_i and P_f for each field were determined from ten 3 × 3-m sampling grids selected systematically and georeferenced for site-specific resampling. SCN populations exhibited an average decline of 42% in 2009, 60% in 2010 and 56% in 2011. Two PCs explained 92% of the total variability in the dataset with the first and second component accounting for 60.5 and 31.5%, respectively. The first PC was largely represented by texture, suggesting that while sand amount increases that of silt and clay decreases. Compared with silt and clay, sand was the textural constituent that showed a stronger association to SCN P_i or P_f (eigenvector values for P_i and P_f = 0.29 and 0.21, respectively and correlation coefficients = 0.51 for P_i and 0.37 for P_f, P < 0.05). The present report is the first assessment of SCN population reduction after corn rotation in commercial production fields in Nebraska, and

also the first description of the relationship of soil texture and SCN population before and after rotation. The PC results indicate that the three soil textural constituents might be considered as a single measure of association when using them as an input in analyses involving texture and SCN population relationships.

Potential for the *Magnaporthe oryzae* Gray Leaf Spot turfgrass pathogen to infect wheat in the U.S.

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Phytopathology 103(Suppl. 3):S3.8

Wheat blast caused by the pathogen *Magnaporthe oryzae* (*Triticum* pathotype; *Mot*), is an emerging fungal disease in South America and is of regulatory concern to the U.S. First reported in Parana, Brazil in 1986, the disease has spread to 90% of Brazil's wheat growing regions as well as Paraguay, Bolivia and most recently, Argentina. In some years, losses of over 30% were reported nationally with some individual fields reporting 10 to 100% loss. In 2011, a single wheat head with blast symptoms was found in a University of Kentucky wheat plot in Princeton, Kentucky. Molecular characterization revealed that this isolate was a local pathotype of *M. oryzae* that causes grey leaf spot of *Lolium perenne* (*Mol*). Previously it has been reported that *Mol* and *Mot* pathotypes infect both *Triticum* spp and *Lolium* spp when artificially inoculated. This was the first report of *Mol* infecting wheat under natural conditions in the U.S. *Magnaporthe oryzae* is a species complex reported on many gramineous hosts in the family Poaceae, including the genera *Oryza*, *Triticum*, *Lolium*, *Festuca*, *Setaria*, *Digitaria*, *Echinochloa* and *Stenotaphrum*. An effort to collect a broad range of *M. oryzae* isolates from a diverse number of host plants is underway. This collection will be used to determine the pathogenic specificity among *M. oryzae* isolates and also will be used for developing *Mot* specific molecular methods to detect and differentiate the pathogen infecting wheat in South America from the endemic forms found on other Poaceae in the U.S.

Rhizobiome responses to new tomato rootstock systems

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Phytopathology 103(Suppl. 3):S3.8

Grafting is an effective technology for tomato growers to reduce soilborne diseases such as *Fusarium* wilt, *Verticillium* wilt, and root knot nematode, and to increase fruit yield. Tomato grafting is rapidly being adopted by high tunnel growers with limited capacity for crop rotation, particularly organic growers who use specialty and/or heirloom varieties as scion. In the case of tomato, inter-specific (*L. esculentum* × wild spp.) hybrid rootstocks are utilized which carry effective resistance to many economically-important diseases and provide added vigor even in the absence of disease. We have initiated a new project to study how the implementation of these rootstocks affects native soil microbes that inhabit the rhizosphere. Our objective is to evaluate the effects of rootstocks on soil and rhizosphere microbial communities, the "rhizobiome", and ultimately to understand the role of the rhizobiome in tomato health. We will evaluate the diversity and composition of soil microbial communities associated with different combinations of scion and rootstock. We will use next-generation DNA sequencing to study the disease resistant rootstock effects on beneficial and pathogenic microbes to support a mechanistic understanding of the effects of grafting and how it can be most useful.

Molecular and cellular responses of date palm roots to salinity stress

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Phytopathology 103(Suppl. 3):S3.8

The ability of date palm (*Phoenix dactylifera* L.) to grow in hot and dry regions of the world makes it an important subsistence crop in most arid areas. Nevertheless, salinity and drought are the main abiotic stresses causing loss of date palm yield. Here we employed the next generation sequencing technology for high-throughput sequencing of date palm transcripts in response to salinity. cDNA libraries, from roots treated with 100 mM sodium chloride and mock treated roots, were sequenced via Illumina HiSeq2000 system. Data analysis identified 1940 genes that are differentially expressed between treated and mock treated roots. MYB transcription factors, protein kinase, E3 ubiquitin ligase, calmodulin, polyamine oxidase and acetyltransferase tran-

scripts are regulated in response to salinity stress suggesting potential roles of these genes in tolerance to salinity stress. Additionally, we examined the cellular responses of date palm tissue to salinity stress using transmission electron microscopy (TEM). Results from TEM show that stressed roots exhibited plasmolysis in cortical cells while epidermal cells did not appear to plasmolyze. Columnar cells in the distal root region might form a barrier to salt intrusion, which would make the older regions of the root less susceptible to salt plasmolysis. Output from this project will help in enhancing our understanding of date palm signaling mechanisms and developing new strategies to control salinity stress.

***Bradyrhizobium japonicum* uses T3SS to suppress soybean defense in early infection stage**

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Phytopathology 103(Suppl. 3):S3.8

Plant pathogens deliver effector proteins into their hosts via Type III Secretion System (T3SS). Some of these T3SS effectors have been shown to function as suppressors of host defense responses. Here we examined the hypothesis that *Bradyrhizobium japonicum* uses its T3SS to deliver Nodulation Outer Proteins (NOPs) that further suppress the host defense. To achieve this goal, soybean root-hair tissues were challenged by a T3SS mutant and a wild-type *B. japonicum* and then soybean genome expression was assessed using RNA-Seq technique. Results show that transcripts related to WRKY and NAC transcription factors, protein kinase, RPM1-interacting protein 4 (RIN4), xyloglucan endotransglycosylase, phenylalanine ammonia-lyase 2 and patellin are being suppressed in root hairs challenged with *B. japonicum* wild type. This information suggests that *Bradyrhizobium japonicum* might suppress the host defense using T3SS to deliver NOPs inside host cells in early infection stage. Additionally we identified novel microRNAs during soybean-*Bradyrhizobium japonicum* interactions, which may suggest a potential role of microRNAs in symbioses.

Changing the way we think and thinking about the way we change

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Phytopathology 103(Suppl. 3):S3.8

Progress is a function of ideas and technologies. Much like the diseases and pests that we study, the dynamics of progress (e.g., rate, direction) are a function of the dynamics of the introduction, establishment, and subsequent spread of ideas and technologies. History indicates that the success or failure of ideas and technologies is not always related to their merit or intent. Thus, it took several decades for sanitation in surgical suites to be adopted and it took several years for public health officials to change the way they thought about the spread of cholera. In both cases, there was a high cost in human lives. The intent for the development of Twitter was as an emergency response tool; its rate of adoption and its use went far beyond the original intent and expectation. Much like our predictive abilities for invasive species, our ability to predict which ideas will establish and how they will spread is less than stellar. Our research technologies today make possible the tremendous generation of knowledge and the rapid evolution of ideas. Our communications technologies and behavior make possible the rapid introduction and spread of those ideas. It is important for scientists to generate knowledge; it is equally important that they disseminate that knowledge so that new ideas and technologies are given the opportunity to either establish and spread or decline to extinction. This symposium will present ideas that are changing the way we think about what we do and technologies that are changing the way we do our work.

Estimating the effectiveness of management strategies to reduce seed degeneration in vegetatively-propagated crops

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Phytopathology 103(Suppl. 3):S3.8

Vegetatively-propagated crops such as potato, sweetpotato, cassava, yam, and banana serve as food security staples for many smallholder farmers in developing countries. Due to the scarcity and/or expense of disease-free seeds, these farmers tend to reuse planting material over multiple generations, resulting in pathogen build up and consequent yield reductions. This problem of 'seed degeneration' is complex and affected by numerous biotic, environmental, economic and human factors. We address this challenge by develop-

ing computational models to evaluate management effects on seed degeneration. The models integrate available management options, such as host resistance, acquisition of clean seed, and on-farm management strategies such as positive selection, and incorporate the role of environmental factors on the choice and success of these management options. They build on existing models of crop virus ecology and evolution, incorporating variability in biological and environmental system elements through stochastic model components. The first model evaluates seasonal time-steps and the second incorporates finer temporal resolution by using vector generation time-steps. The latter also incorporates spatial structure by evaluating the effects of decision making on multiple neighboring farms. These models will then be used to evaluate crop-specific management strategies and develop a decision support system to predict the effectiveness of different management strategies in reducing seed degeneration or in reversing the phenomenon to improve existing seed quality.

Effect of mowing height and seeding rate on rough bluegrass encroachment and brown patch disease severity during tall fescue lawn establishment

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Phytopathology 103(Suppl. 3):S3.9

Roughstalk bluegrass (RB, *Poa trivialis*) is a weed in tall fescue (TF, *Festuca arundinacea*) lawns and may be introduced as a seed contaminant. Selective herbicides are limited, and cultural control strategies would be beneficial, but these practices may also influence development of diseases such as brown patch (BP), caused by *Rhizoctonia solani*, or conflict with overall agronomic best management practices, leading to challenges for integrated control. The objective of this study was to determine the influence of seeding rate and mowing height on BP severity and RB colonization. Plots were seeded in September 2011 with TF at 195, 391, or 586 kg ha⁻¹ with 1.0% RB seed contamination by weight. Mowing treatments (3.8-, 7.6-, or 11.4-cm) were initiated when turf reached designated heights. Brown patch was assessed by visually estimating the percentage of each plot displaying symptoms, and RB was assessed using an intercept grid. There was no mowing height × seeding rate interaction. In July 2012, the 7.6-cm mowing height had greater RB cover than 3.8- or 11.4-cm mowing heights. In September, whole-plots mowed at 7.6-cm averaged greater RB cover than mowing at 3.8-cm. The 586 kg ha⁻¹ seeding rate averaged greater RB cover in September 2012 than seeding at 195 kg ha⁻¹. In November 2012, sub-plots seeded at 391 or 586 kg ha⁻¹ averaged greater RB cover than seeding at 195 kg ha⁻¹. On 5 July, whole-plots mowed at 7.6-cm averaged more BP than plots mowed at 3.8- or 11.4-cm. On 18 July, whole-plots mowed at 7.6 or 11.4-cm averaged more BP than plots mowed at 3.8-cm and sub-plots seeded at 586 kg ha⁻¹ had more BP than sub-plots seeded at 195 kg ha⁻¹. All treatments yielded acceptable establishment of TF, but higher mowing heights and seeding rates generally resulted in increased RB colonization and BP incidence.

RNAi: Transfection to prevent infection

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Phytopathology 103(Suppl. 3):S3.9

RNA interference (RNAi) has become a valuable tool for researchers studying gene validation and reverse genetics. For plant protection, there are increasing numbers of reports demonstrating the effectiveness of gene silencing as a method to control pest and pathogens. We have been working on a number of systems that utilize gene silencing for plant protection. Two of these systems include virus resistance in wheat and nematode protection in soybean. In wheat, research has been focused on developing resistance to *Wheat Streak Mosaic Virus* and *Triticum Mosaic Virus* by creating RNAi vectors targeting various viral genes and expressing these constructs in transgenic wheat. In particular we have identified independent transgenic lines expressing siRNAs targeting the coat proteins (CP) of these two viruses. The TriMV-CP lines have been advanced to the T3 generation and still display a resistant phenotype. The WSMV-CP lines have been advanced to the T6 generation. Both lines are being introgressed into the winter variety 'Overly'. In soybean, RNAi is being used to develop resistance to soybean cyst nematode and root knot nematode. The strategy of these projects is to target genes of the nematodes that are required for reproduction or fitness and express them in soybean. As the nematode parasitizes the roots they ingest the siRNA produced by the soybean and the target genes are down-regulated in the nematode. Over thirty SCN genes have been identified and tested in a composite "hairy root" SCN bioassay. Results performed on the composite plants indicated that seven RNAi constructs resulted in significant reduction for SCN eggs, while the other constructs did not have significant effects. Efforts on creating stable transgenic events, limitations and future direction of these projects will be discussed.

Human Pathogens on Plants: Novel strategies to reduce outbreaks

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Phytopathology 103(Suppl. 3):S3.9

Healthy and safe food can be produced in healthy ecosystems characterized by integrity of nutrient cycles and energy flows, stability and resilience to disturbance, and suppression of pathogens and pests. Healthy ecosystems have diverse microbial communities cycling through successive habitats in association with food webs. For example, microbial communities would transit from soil to rhizosphere and endosphere of plants, animals, manure, and back to soil. A healthy, biologically diverse soil would give rise to a diverse community on roots, in plants and animals resulting in greater systemic resistance and better immune systems, respectively. Evidence for microbial cycling and a positive relation between microbial diversity and health is increasing. Examples are given of gfp-marked *Salmonella* and *E. coli* O157:H7 transiting from animal feed to guts, manure, soil, plants, plant eating animals and their excrements. Risk factors for survival of these pathogens in manure, soil and water are presented. Survival was enhanced by high carbon and nitrogen concentrations per unit microbial biomass. Low activity and diversity of competing microorganisms and low oxygen levels were also risk factors. Movement of *Salmonella* in lettuce and tomato plants was reduced at greater endophytic diversities. Survival inside tomato leaves was reduced in plants grown in organic versus conventional soils, with lower N, higher OM, Ca and Mg contents in organic soils and higher microbial diversity in plants in those soils. Movement through phloem into tomato fruits and seeds was observed only for plants in conventional soil. However, the probability of internalization is low. A microbial risk assessment model for *E. coli* O157:H7 indicated that 1 in 10,000 lettuce heads could be internally contaminated; this risk would be about 9 times higher in conventional than organic production systems when manure is applied in both. Thus, sustainable farm nutrient management can minimize risks of pathogen survival and spread.

Genetic and aggressiveness variation in *Sclerotinia sclerotiorum* isolates from dry bean fields in Brazil

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Phytopathology 103(Suppl. 3):S3.9

Sclerotinia sclerotiorum the causal agent of white mold, causes severe yield losses in dry bean. Disease resistance is a management strategy with no costs to growers. The selection of resistance bean plants requires knowledge of isolate variation. The objectives of this study were to characterize 25 *S. sclerotiorum* isolates from Brazilian dry bean fields using microsatellite markers, mycelial compatibility groups (MCGs) and aggressiveness. Eight fluorescently-labelled microsatellite primer pairs were used to identify polymorphisms among the *S. sclerotiorum* isolates. To determine the MCGs, each possible pair of isolates was grown in a side-by-side pairing on a plate of DS medium. Compatible isolates merged to form one colony while incompatible isolates formed a barrage line of dead cells. For the aggressiveness test a straw containing a plug of agar and fungal mycelium was placed over a cut stem at 21 days post-planting and after 8 days were rated for symptoms. Data from microsatellite profiles grouped the isolates into four clusters using Euclidean distance algorithm. Among the isolates tested, seven MCGs were identified. Only 57% of MCGs were sampled frequently over multiple locations and 43% were unique to locations. There were significant differences among isolates in aggressiveness. The Unaí isolate was significantly more aggressive than isolates from other locations, whereas the Viçosa isolate was significantly less aggressive than those from the other locations. For identification of the highest levels of resistance the Unaí isolate should be used.

Temporal evaluations of the wheat stem rust infection process on barley cultivars via relative q-PCR

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Phytopathology 103(Suppl. 3):S3.9

The infection process of the wheat stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, in *Hordeum vulgare* L. (barley) is often observed as a mesothetic infection type at seedling stages; creating inherent error when using traditional phenotyping methodology. Furthermore, cultivars containing the same resistance genes often show great variation in the level of resistance provided to the same isolate. The use of quantitative real time polymerase chain reaction (q-PCR) to determine the amount of fungal DNA relative to a host specific gene can allow researchers to evaluate the relationships of fungal development and host resistance on a temporal basis. Five barley cultivars with defined genotypes and infection types to *P. graminis* race MCCF were

assessed using a relative q-PCR approach over five time points. The susceptible cultivar Steptoe, resistant cultivars containing only Rpg1 (Beacon, Morex, and Chevron), and the resistant cultivar Q21861 containing Rpg1 and the rpg4/Rpg5 complex were evaluated using the traditional 0–4 rating scale at 12 days post inoculation (DPI), fluorescence microscopy at 24 and 48 hours post inoculation (HPI), and q-PCR immediately after inoculation, 24 HPI, 48 HPI, 6 DPI, and 12 DPI. The q-PCR assay was able to observe statistical differences ($P < 0.05$) in fungal development as early as 24 HPI. Fungal

development observed using fluorescence microscopy displayed the same phenotypic pattern observed using the q-PCR assay. Furthermore, the q-PCR assay was able to separate the cultivars by their expected level of susceptibility at 12 DPI using traditional phenotyping as early as 6 DPI; three days prior to the formation of visually observable pustules. The fungal development occurring at 24 and 48 HPI is vastly different than what was expected at 12 DPI, indicating potential host genotype contributions related to basal defense during the *P. graminis* infection process.