Proceedings of the North Central American Phytopathological Society Meeting

June 11-13, 2014

UWEX Pyle Conference Center – 702 Langdon Street – Madison, WI 53706

Coordinators of Proceedings

Amanda Gevens, Tyler Engel, Amy Charkowski, Mehdi Kabbage, An Nguyen

---

Symposium I

Impact of Genomic Research and Advancements on Production Agriculture: Is Science Translating to the Field

Coordinator: Dr. Amy Charkowski, Professor, UW-Plant Pathology

Moderator: Ms. Alejandra Huerta, Graduate Research Assistant, UW-Plant Pathology

The Phytobiomes Initiative

Beattie, G.A., *Iowa State Univ., Plant Pathology & Microbiology, and J.E. Leach, Colorado State Univ., Fort Collins, CO*

The phytobiome is the entire microbial community that is in, on and adjacent to plants. This includes the bacteria, archaea, fungi, nematodes, oomycetes and viruses. The ability to study the phytobiome as a system will reveal how this community influences, and is influenced by, the plant. Recent advances in high-throughput sequencing, computational biology, and many "omics" technologies are enabling exploration of the composition, function and activities of the cultured and uncultured members of these communities. The Phytobiomes Initiative is a new advocacy focus of the APS Public Policy Board aimed at generating support and activities to achieve a comprehensive understanding of phytobiomes. Foundational and applied knowledge is critical for optimizing phytobiomes to help increase agricultural productivity for sustained global food security. This talk will focus on examples of scientific questions encompassed within phytobiomes research, such as how the phytobiome influences plant tolerance to stresses and how the host shapes the phytobiome. The talk will also focus on the relevance of phytobiome studies to strategies for controlling plant diseases, improving crops, addressing environmental challenges, and assuring food safety. The goal of the talk is to help introduce the Phytobiomes Initiative and highlight its role in moving us beyond a focus on individual microbes in plant health and productivity to a focus on the networks of interacting microbes that are influencing, and being influenced by, plants and the significance of this systems knowledge to our future global food security.

Insights and Applications of the SolCAP Genome-Wide SNP Array

***Douches, D.**, Buell, C.R., Coombs, J., Manrique, N.C., Massa, A., Felcher, K., Zarka, D., Michigan State Univ., Potato Breeding & Genetics*

The Solanaceae Coordinated Agricultural Project (SolCAP) was supported by the USDA-NIFA from 2008-2014. Through innovative research, education and training, the SolCAP project focused on translating genomic advances to US tomato and potato breeding programs. A major impact from SolCAP has been the widespread use of the SolCAP 8303 Infinium Potato SNP Array as a common marker platform, with over 25,000 samples assayed by collaborators worldwide. The genome-wide SNP Array has provided a powerful new tool for potato breeders to evaluate potato clones and investigate genetic questions. Population structure and diversity analysis has been conducted on the diversity panel of 250 North American potato varieties and breeding lines. The diversity panel was also used to investigate allele frequency and heterozygosity in North American potato germplasm. At MSU, four tetraploid and three diploid mapping populations have been genotyped and phenotyped for economically important traits. The SNPs in these mapping populations have been useful in studying genetic phenomena including distorted segregation and double reduction. The SNP Array has been used to study 25 potato wild species from the core collection from the US Potato Genebank, as well as heterozygosity in selfing diploid *Solanum chacoense* species. Another application of the SNP Array is for fingerprint analysis, where tetraploid varieties and breeding lines are uniquely distinguished by over 1,000 SNP markers using tetraploid dosage, from a database of over 300 different potato clones (including closely related clones).

Translation of Molecular Biology and Physiology to Improve Bioenergy Traits in Rice and Sorghum

***Jahn, C.E**., Tanger, P., Turner, M.F.S., Leach, J.E., Colorado State Univ., Bioagricultural Sciences & Pest Management, and Kirkwood, J.S., Heuberger, A.L., Proteomics and Metabolomics Facility, Colorado State Univ.*

Sustainable energy production from cellulosic feedstock requires a comprehensive approach to maximize plant biomass production. For lignocellulosic energy production to become economically viable, efforts must be made to both improve feedstock conversion and also refine plant breeding targets to better meet needs of these processes. This may be achieved through development of crops with tailored cell-wall composition or engineering of plants to produce desirable biochemicals. Phenotyping is a well-known bottleneck in breeding efforts and data collected in controlled environments is difficult to translate to the field. Our research seeks to characterize agronomic bioenergy traits across diverse environments to determine phenotypes that are rapidly identifiable and robust at the field level. We used *Sorghum bicolor* (sorghum) and *Oryza sativa* (rice); model C_4 and C_3 grass species, respectively. Both represent multipurpose crops which can serve food, forage or biofuels markets. Sorghum and rice also display extensive phenotypic variation in biomass quantity and quality traits. We grew diverse sets of germplasm for both species in multiple years and locations. Here we report bioenergy-related parameters including cell-wall composition, digestibility, and sugar release, as well as morphological, physiological and biochemical traits. We found significant variation among genotypes and across environments for most traits and efforts to improve bioenergy traits must examine both stem and leaf tissues separately as they may be under separate genetic control. Our data suggest greenhouse studies may overestimate bioenergy potential of cellulosic biomass, although trends remain consistent between controlled and field conditions and may still prove useful predictors of field performance.

Impact of Genomic Research and Advancements in Production Agriculture: Pathogen Genomics and IPM via Improved Diagnostics

***Gevens, A.J.**, and Charkowski, A.O., University of Wisconsin-Madison, Plant Pathology*

Globally, plant diseases caused by fungal, bacterial, viral, and other pathogens in agricultural crops can lead to major losses. Effective control responses must be anchored in accurate diagnosis of disease and on knowledge of pathogen distribution. Multiple approaches, including techniques based on biological properties of host and/or vector interaction, and techniques based on intrinsic pathogen properties such as nucleic acid and coat protein, are utilized for detection and identification. A hallmark of Integrated Pest Management (IPM) is its systems approach using current, comprehensive information on pathogen life cycles and their environmental interactions, and available disease control methods (cultural and chemical) by the most economical means and with the least harm to people, property, and the environment to manage plant disease. Advances in genomics allow us to now understand an organism or population on a genome-wide scale. How can genomics be used in an inclusive manner with IPM to improve disease control? Next generation sequencing (NGS) platforms have facilitated the collection of DNA sequence data and have been useful for genomic research on non-model organisms. NGS can aid in rapid discovery and description of polymorphisms, such as fungicide resistance, within a population and may be coupled with spatiotemporal disease data to make enhanced, geographically-specific and appropriately-timed management decisions. Additionally, genomic exploration leads to resolution of a greater number of specific markers for traits of interest, making feasible high throughput and simultaneous plant disease diagnosis of numerous pathogens. In particular, diagnostics on asymptomatic plant tissues such as true seed or propagative tissues, is of great interest and value. Application of genomic tools to improve diagnostics and management in commercial production requires vetting to ensure that protocols are efficient and validated for practical use and that results are repeatable and robust given the complexity of some of our most challenging plant diseases.

Symposium II

Student Oral Presentations – Competition

Coordinator: Dr. Amanda Gevens, Assistant Professor, UW-Plant Pathology

Moderator: Dr. Tamra Jackson-Ziems, Associate Professor, Univ. of Nebraska-Lincoln-Plant Pathology

Using Geostatistics to Study Soilborne Pathogens in Michigan Potato Fields

***Steere, L.**, Rosenzweig, N., Kirk, W., Michigan State University*

In 2012, a team comprised of growers and university researchers was established to address the issue of declining yields and decreased tuber quality in Michigan potato (*Solanum tuberosum*) production. The goals of the research were 1) to better understand soil-borne pathogen inoculum levels in potato fields, 2) To better understand the soil biology and quantify soil microbial diversity and 3) to make correlations between yield and soil biological factors. In addition to interactions among soil properties and microbial diversity, the research team incorporated the use of geostatistics and geographic information systems (GIS) to create predictive maps of diversity, soil pathogen populations and yield of entire fields from the sample points. Twenty soil sample points were collected from 26 fields prior to potato planting in 2013 and each sample point was assessed for soil characteristics, *Verticillium dahliae* colony forming units, and soil microbial diversity. At harvest, yield (t/ha) was evaluated within each sampling grid and tubers were assessed for incidence and severity of potato common scab (*Streptomyces scabies*). After all data were

collected, each field was assessed individually for spatial continuity and variability of the sample points using geostatistical parameters. Information was then interpolated using various geostatistical mapping methods and, statistical correlations were made. The procedures and methods developed during this study will become a useful tool for understanding microbial interactions as well as visualizing pathogen levels as part of an integrated pest management system.

Hydroxycinnamic Acid (HCA) Degradation Contributes to *Ralstonia solanacearum* Virulence by Eliminating Plant Defense Molecules

Lowe, T.*, *Ailloud, F., Fochs, B., Allen, C., University of Wisconsin-Madison*

The xylem-colonizing plant pathogen *R. solanacearum* causes bacterial wilt disease. Plants produce antimicrobial hydroxycinnamic acids (HCA) to combat pathogens. Tomato plants infected with *R. solanacearum* express genes involved in HCA biosynthesis. To determine whether HCA degradation is important during disease, we constructed a Δfcs mutant, which lacks the first enzyme in HCA degradation. When inoculated via a naturalistic soil soak method, the Δfcs mutant displayed delayed symptom development on tomatoes. We investigated several hypotheses why HCA degradation contributes to virulence: (1) carbon acquisition, (2) removal of phenolic barriers (e.g. lignin) that restrict pathogen movement, and (3) breakdown of antimicrobial compounds. Although HCA degradation allows growth on HCAs, Δfcs did not have a growth defect in root exudate or xylem sap, indicating that HCAs are not a significant *in planta* carbon source. *R. solanacearum* infections did not affect quantity or distribution of lignin in tomato stem. Δfcs bacteria systemically colonized the host xylem at the same rate as wildtype. Therefore HCA degradation does not limit phenolic barriers in the host and does help *R. solanacearum* spread in the plant. The Δfcs bacteria are more susceptible to toxicity of the HCAs caffeate and p-coumarate in a growth inhibition assay. Results suggest HCA degradation protects *R. solanacearum* from the toxicity of plant chemicals.

Seed Treatments, In Furrow and Early Foliar Treatments for Control of Seed-borne *Phytophthora infestans*

Dangi, S.*, *Shafer, R., Somohano, P., Kirk, W., Michigan State University*

The efficacy of nine commercially available fungicides against potato seed-borne late blight caused by different genotypes of *Phytophthora infestans* (US-8, US-22, US-23 and US-24) was evaluated in controlled environment (CE) chambers and in the field. After inoculation of cut seed pieces (immersion in zoospore/sporangium inoculum suspension for 30 min) and application of fungicides plant stand (%) was recorded over a 6-week period after planting and the Relative Area Under the Emergence Progress Curve (RAUEPC) was determined. In CE experiments, all treatments were significantly different from the inoculated check in both years at the final plant stand evaluation. Mandipropamid (single and double rates), flutolonil + mancozeb, fludioxonil + mancozeb and mandipropamid + mancozeb were not significantly different from the not-inoculated check in terms of plant stand and RAUEPC. There were no significant differences among genotypes of *P. infestans* on plant stand and RAUEPC in 2012 but in 2013 US-22 inoculated seed treatments had a lower plant stand relative to the other genotypes. All the treatments had lower disease incidence compared to the inoculated check. In the field experiment, all the treatments except the foliar application of mefenoxam + chlorothalonil (applied at 95% emergence) had significantly greater plant stand and RAUEPC in comparison to the inoculated check. Responses of some treatments, relative to the not-inoculated not-treated check, indicated that some treatments enhanced emergence rate in 2012. The period between inoculation and seed piece treatment was increased in 2013 by 12 h, resulting in higher disease severity and lower plant stand compared to 2012. No treatments except mandipropamid + mancozeb (single and double rates), fludioxonil + mancozeb and mancozeb

were significantly different in plant stand and RAUEPC in comparison to the inoculated check in 2013. These results demonstrated the efficacy of some commercial fungicides to manage seed borne late blight.

The Pathogenicity of the Interspecific Hybrids Between *Fusarium fujikuroi* and *F. proliferatum* Towards White Pearl Onion

Mohamed Nor, N.M.I.*, *Toomajian, C., Todd, T., Leslie, J., Kansas State University*

Interspecific hybrids offer opportunities to study the segregation of pathogenicity between species. Transgressive segregation of pathogenicity factors can result in strains that are more or less aggressive than the parent. *Fusarium proliferatum* and *F. fujikuroi* are very closely related. We collected hybrid progeny from crosses between the two species under laboratory conditions. 432 viable ascospores (progeny) were collected with no more than 20 progeny per perithecium. Amplified Fragment Length Polymorphisms (AFLPs) were used to fingerprint the progeny. Onions were mechanically wounded and inoculated with agar plugs from colonies growing on potato dextrose agar (PDA). A grading scale based on symptoms observed 14 days post-inoculation was used to assess pathogenicity. *F. proliferatum* is pathogenic to onions and *F. fujikuroi* is not. Three phenotypic pathogenicity traits were observed on onions: (i) external lesions, (ii) internal lesions, and (iii) blisters. Of 32 possible phenotypes, only 14 were present amongst the progeny. Forty percent of the progeny had the same phenotype as the *F. proliferatum* parent, 19% had the same phenotype as the *F. fujikuroi* parent, and 41% had a novel phenotype distinct from that of either parent. Some of the progeny were more aggressive pathogens than either parent. Association of markers on the physical map of the genome with these phenotypic traits will aid in the identification of genes that encode macromolecules critical for these fungi to pathogenize onions.

Engineering Resistance to Potato Virus Y in Various Potato Varieties

Arcibal, E.*, *Jahn, M.M., Jiang, J., Rakotondrafara, A.M., University of Wisconsin-Madison*

Potato virus Y (PVY) remains a persistent problem in potato production. An important contributor is the popularity of several varieties, including Russet Norkotah and Silverton, that exhibit mild or no symptoms to PVY. This makes detection of the virus difficult, causing these varieties to act as PVY reservoirs. PVY incidence has been exacerbated with the emergence of viral strains that cause tuber necrosis, as well as the presence of new invasive vectors. Major efforts have been made to development effective strategies to reduce PVY incidence, including breeding for PVY resistance and the release of certified PVY free seed potato lots. Genetic engineering is a powerful tool for trait introgression that maintains the characteristics of original cultivars. We identified the potato gene closely related to the natural PVY resistance genes in tomato and pepper and modified it to confer resistance. The resistance gene encodes the Eukaryotic Initiation Factor 4E (eIF4E), an important susceptibility factor in the viral life cycle. We generated transgenic Russet Norkotah, Atlantic and Silverton potato varieties by modifying the endogenous potato eIF4E with mutations similar to that of the pepper resistance homolog. Our goals are to: 1) Screen the transgenic lines for resistance against PVY:O, N:O, and NTN strains; 2) Test PVY spread in the developing tubers; 3) Assess heritability of the eIF4E- resistance phenotype by crossing with a wild type. Initial screens revealed that our transgenic Russet Norkotah and Atlantic lines tested negative to PVY strains O and N:O accumulation both in local and systemic leaf tissues.

A Microbiological Examination of *Erwinia amylovora exopolysaccharide* Ooze, the Most Important Component of Fire Blight Disease Epidemiology

Slack, S.*, *Sundin, G., Michigan State University*

Erwinia amylovora is the causal agent of fire blight, the most damaging bacterial disease of apple and pear trees. *E. amylovora* is mainly disseminated through ooze, a viscous mass of bacterial cells and exopolysaccharides which is exuded from infected tissue. We conducted a microbiological assessment of ooze, characterizing *E. amylovora* populations associated with ooze droplets from field-infected trees, and used microscopy techniques to analyze ooze extrusion from apple stems. For population studies, we examined 201 ooze droplets emerging from inoculated shoots of apple ('Jonathan'). These droplets averaged 2.7 microliters in volume and harbored an average of 1.0x109 and a median size of 1.3x108 *E. amylovora* cells per microliter. The color of the ooze drops ranged from white to dark red; yellow drops were the largest and red drops harbored the highest *E. amylovora* populations. *E. amylovora* populations were also assessed from 1-cm stem sections on either size of an ooze drop to determine if excessive bacterial growth contributed to ooze extrusion. To analyze the extrusion process, we used scanning electron and confocal laser-scanning microscopy techniques. Microscopic examination of apple stem tissue at sites of ooze extrusion revealed that bacteria were not escaping the plant through natural openings. Instead, erumpent mounds, indicative of internal pressure buildup, were visualized suggesting that stem wounds with exuding ooze had been initiated by *E. amylovora* cells. Through these ooze studies; the base-knowledge of *E. amylovora* dispersal is increasing, which could have future impacts in fire blight disease management.

Symposium III

From Host Recognition to Transmission: Advances in Mechanisms of Infection

Coordinators: Dr. Mehdi Kabbage, Assistant Professor, UW-Plant Pathology & Dr. Aurelie Rakotondrafara, Assistant Professor, UW-Plant Pathology

Moderator: Mr. Jose Pablo Soto-Arias, Graduate Research Assistant, UW-Plant Pathology

Biochemical Defenses of Leaf Surfaces: New Strategies for Disease Control

Shepherd, R., *Phyllotech LLC, Madison, WI*

Leaf surfaces comprise an aerial frontline in plant-microbe interactions. The identification of diverse proteins that are present in leaf surface environments underscores the complexities underlying pathogen infection. In *Nicotiana tabacum*, glycoproteins termed phylloplanins are biosynthesized in specialized epidermal structures called short glandular secreting trichomes and are secreted to leaf surfaces where they act as first-point-of-contact deterrents to pathogen establishment. The plant pathogen and leaf surface epiphyte *Pseudomonas syringae* utilizes acyl homoserine lactones as signaling molecules to coordinate gene expression in a cell-density dependent manner, and produces AHL-degrading enzymes that might be utilized to degrade the signaling molecules of other bacterial species. Our current research programs to develop protein-based pathogen controls and trichome bioproduction systems allude to exciting advances in our abilities to modify phylloplanar environments, and reveal an abundance of promising targets for downstream commercialization pursuits.

Final Transmission: Exploiting the Specificity of Virus-Vector Interactions for New Disease Control Strategies

***Whitfield, A.**, Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS*

The majority of viruses that infect plants are transmitted from one host to another by arthropod vectors. Our research goal is to identify viral and vector determinants of transmission and characterize the direct effect of virus infection on vectors. Defining the molecular determinants of a plant virus–vector interaction enables the development of novel virus control strategies that aim to specifically disrupt the interaction. Our first approach towards breaking the disease transmission cycle involves blocking virus acquisition by insect vectors. For example, Tomato spotted wilt virus (TSWV) acquisition is mediated by the interaction between the virus membrane glycoprotein GN, which serves as a viral attachment protein, and the thrips midgut. We generated transgenic tomato plants expressing a soluble form of GN and found that an initial reduction in virus infection of the larval insect midgut resulted in a significant decrease in virus titer and transmission over the life-span of the vector. Our second approach centers on identifying vector proteins that interact directly with viral proteins. We have identified thrips and planthopper proteins that bind to viral glycoproteins of tospoviruses and rhabdoviruses, respectively. As virus binding-proteins are characterized in insect vectors, we can develop new strategies to prevent virus acquisition and dissemination in vectors. Lastly, we have developed transcriptome resources for vectors, and we are using these sequences for RNA interference (RNAi) in insect vectors. Effective RNAi will enable the development of genetically modified plants that specifically target proteins required for vector development, feeding, or virus transmission. We envision these strategies being further developed and incorporated into existing integrated crop management programs as a valuable component of a sustainable food production program.

Evolution and Engineering of Signaling Pathways Controlling Plant-Microbe Symbioses

***Ané, J.M.,** Dept. of Agronomy, University of Wisconsin-Madison, WI*

Beneficial associations between plants and arbuscular mycorrhizal (AM) fungi play a major role in terrestrial environments and in the sustainability of agroecosystems. Proteins, microRNAs and small molecules have been identified in model angiosperms as required for the establishment of AM associations and define a symbiotic 'toolkit'. Using a comprehensive phylogenetic analysis of these components in the plant lineage we characterized a step-by-step appearance of this ‘toolkit’, with some components predating the first land plants whereas some others appeared recently in flowering plants. In addition, we found that independent losses of the AM symbiosis in several flowering plant lineages are systematically correlated with the loss of the whole ‘toolkit’. Using this correlation and a comprehensive comparison of the sequenced angiosperm genomes we identified new candidate genes potentially required for AM associations. The symbiotic ‘toolkit” has been recruited for other interactions such as the rhizobia–legume symbiosis. Surprisingly, colonization assay of rice mutants affected in the symbiotic ‘toolkit’ with Rhizobium sp. IRBG74 revealed that rhizobia-monocot symbiosis could also rely on this genetic pathway. Rhizobium sp. IRBG74 has been found able to induce rice gene expression in a similar manner than AM fungi. Moreover, diffusible signals produced by Rhizobium sp. IRBG74, like signals produced by AM fungi, triggered peri-nuclear calcium spiking in rice epidermal. These results indicate that Rhizobium sp. IRBG74 colonizes rice via the symbiotic ‘toolkit’ by mimicking AM fungi signals.

Roles for Oxalate Synthesis and Catabolism in *Sclerotinia sclerotiorum* Pathogenesis

***Rollins, J.**, Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL*

Accumulation of oxalic acid (OA) during colonization is a hallmark of the necrotrophic pathogenesis of *S. sclerotiorum*. This simple dicarboxylic acid has functions including divalent cation chelation, ambient pH

modulation, xylem occlusion, guard cell misregulation, elicitation of host programmed cell death, and modulation of the ambient redox environment. Yet, the dynamics of OA accumulation during colonization are unknown. We focused on understanding the metabolism and regulation of OA accumulation by *S. sclerotiorum*. OA accumulation is regulated by ambient pH. Neutral pH promotes and acid inhibits accumulation across nutritional conditions. pH-responsive dynamics are largely under the regulatory control of Pac1, a C2H2 Zn-finger transcriptional regulator. Loss of function pac1 mutants accumulate OA at a reduced rate and virulence. Gain of function pac1 strains accumulate wild-type levels of OA but do so independent of ambient pH conditions and exhibit a subtle decrease in virulence relative to wild type. Characterization of the oah1-encoding gene demonstrated its Pac1-dependent, pH-responsive transcript accumulation is a requirement for OA production. The loss-of-function oah1 gene deletion mutants exhibit attenuated virulence but limit colonization and produce a subset of symptoms. Factors other than OA may condition compatibility and OA may play a primary role in the spreading lesion and colonization phases. Consistent with the hypothesis that OA is of less importance and detrimental to the early stages of host-pathogen compatibility, an oxalate decarboxylase gene with transcript accumulation tightly coupled to compound appressorium development was identified. Deletion of this odc2 gene results in a cuticle penetration defect linked to compound appressorium development and function. Wounding of host tissues bypasses the loss of Odc2 function and restores wild-type symptoms. The dynamics of OA accumulation involves environmentally-regulated OA synthesis via oxaloacetate as well as developmentally-timed catabolism of OA. Dynamically regulated OA accumulation plays an important role in compatibility and necrotrophic success.

Abstracts of Poster Presentations

Coordinator: Dr. Amanda Gevens, Assistant Professor, UW-Plant Pathology

Asterisk by name of first author indicates participation in the Student Poster Competition.

1

Reaction of Global Spring Wheat Genotypes for Resistance to Bacterial Leaf Streak

Abdullah, S.*, *Ali, S., Singh, P., Glover, K., Gonzalez, J., South Dakota State University*

Bacterial leaf streak (BLS) is an important foliar disease of wheat in the US northern Great Plains. The disease is caused by the bacterium *Xanthomonas translucens* pv. *undulosa* (Xt)). A majority of the commercial cultivars grown in the region are susceptible to BLS. It is essential to search for sources of resistance for the development of BLS resistant cultivars. In this study, 286 wheat genotypes developed by CIMMYT were evaluated for their reaction to BLS. Three leaves of each genotype at the two-leaf seedling stage, raised in containers in greenhouse, were infiltrated with a bacterial cell suspension (1x108 Cfu/ml) with a needleless syringe. Infiltrated leaf area was marked with a permanent marker. The infiltrated leaves were rated for BLS reaction 10 days post-infiltration based on the lesion length expansion (0 = resistant; < 1 cm = moderately resistant; 1-1.9 cm = moderately susceptible; and >2 cm = susceptible) from the marked infiltrated area. Of 286 genotypes evaluated, eleven were resistant; 142 were moderately resistant; 113 were moderately susceptible; and twenty were susceptible. The results indicate that CIMMYT wheat material has diverse response to BLS, and the resistant genotypes can be utilized as a source of resistance for development of BLS resistant wheat cultivars in the northern Great Plains.

2

Evaluation of Soybean Genotypes and Identification of Quantitative Trait Loci (QTL) for Resistance to *Fusarium graminearum*

***Acharya, B**.*, Lee, S., Wickramasinghe, D., Michel, A., McHale, L., Rouf Mian, M.A., Dorrance, A.E., The Ohio State University*

Recently, *Fusarium graminearum* was established as a primary pathogen of soybean causing seed rot and seedling root rot in the USA. Initial symptoms start as water soaked lesions which turn light brown or have pink discoloration at the later stages. The objective of this study was to evaluate soybean genotypes for resistance towards *F. graminearum* and to characterize resistance using recombinant inbred lines (RILs) of two soybean populations: 'Wyandot' x PI567301B and 'Conrad' x 'Sloan'. The parents and the RILs of the mapping populations were evaluated for resistance using the roll towel assay in a randomized complete block design or augmented randomized incomplete block design. The populations were genotyped using Infinium BARCSoySNP6K BeadChip array. Linkage maps and QTL analysis were constructed using JoinMap4 and MapQTL5. Thirty genotypes had a disease severity index of less than 50% to *F. graminearum*. In the Wyandot x PI 567301B population, one major QTL on chromosome 8 and two minor QTL on chromosomes 6 and 10 were identified. In the Conrad x Sloan population, one major QTL was identified on chromosome 19 and one minor QTL on chromosome 10. The QTL identified in the study will be useful in developing cultivars with resistance to *F. graminearum*.

3

Developing Germplasm Resources to Identify the Genetic Basis of Resistance to Common Scab in Potato

***Allen, C.*,** Charkowski, A.O., Jansky, S., University of Wisconsin-Madison Horticulture, USDA*

Common scab, caused mainly by the soil-borne bacterium *Streptomyces scabies*, produces lesions on potato tubers, reducing tuber quality and profitability. Methods to manage common scab are often expensive, impractical, and can be ineffective. Therefore, creating cultivars that are resistant to common scab has been cited as the best method to control the disease. 524-8, an inbred diploid line of the wild potato species *Solanum chacoense*, exhibits significant and consistent resistance to common scab. M6, a *S. chacoense* inbred line with high genetic similarity to 524-8, is susceptible to common scab. These two closely related lines offer a great opportunity to examine the factors that contribute to resistance or susceptibility to common scab. Recombinant inbred lines (RILs) of 524-8 x M6 are being created by self-pollinating successive generations of the F2 population to achieve homozygosity. The RILs will then be phenotyped and genotyped to identify the genetic regions associated with scab resistance.

4

Development of a New Approach to Characterize Pathogen Virulence of *Pyrenophora tritici-repentis*, the Causal Agent of Wheat Tan Spot

***Ameen, G.*,** Liu, Z., Faris, J., Mergoum, M., North Dakota State University*

The ascomycete *Pyrenophora tritici-repentis* (Ptr), the causal agent of tan spot of wheat, is a destructive disease in many wheat-growing regions. The pathogen produces at least three host selective toxins (HSTs) that interact with their corresponding host sensitivity genes in an inverse gene-for-gene manner to cause disease. However, more and more evidence has suggested that this system is not merely based on these three HSTs, but involves additional uncharacterized HSTs and possibly other genetic factors. Due to

the homothallic nature of the fungus, it is difficult to develop segregating Ptr populations through sexual mating, but such populations would be extremely useful for characterizing fungal pathogenicity/virulence factors using the power of genetic mapping. In this work, we developed mating type gene knockouts in Ptr and subsequently showed that the deletion of either one of the mating type genes (MAT1-1/MAT1-2) leads to sterility of the fungus. Crosses between the two knockout strains with the opposite mating type were performed and resulted in the production of mature and functional ascopores. A small fungal population has been developed from the cross between the two strains and characterization of the fungal progenies is underway. This work will establish an effective way to further identify Ptr virulence factors that are undetectable using current methods.

5

Assessment of Resistance in Soybean Towards *Pythium ultimum* var. *ultimum* and *Pythium ultimum* var. *sporangiiferum*

Balk, C.*, *Fitzgibbon, T., Huzar Novakowiski, J., Erye, M., Dorrance, A.E. The Ohio State University, Department of Plant Pathology*

In Ohio, seedling blight caused by oomycetes is an annual problem in crop production. More than 25 different species of *Pythium* have been identified that contribute to seed and seedling loss in Ohio; which is economically detrimental to soybean. Several factors have been proposed that may contribute to the incidence of *Pythium* including long term no-till production, changes in seed treatments, and environmental conditions that favor infection that occur immediately following planting. Host resistance and seed treatments are two management strategies that could be deployed for these pathogens. Over 300 soybean genotypes were screened for partial resistance with one isolate of *Pythium ultimum* var. *ultimum* (Miami 1-3-7) and one isolate of *Pythium ultimum* var. *sporangiiferum*(Will 1-6-7). Overall, there were 15 genotypes conferring high resistance (Root score of less than 2.0) to *Pythium ultimum* var. ultimum and *Pythium ultimum* var. *sporangiiferum*, respectively. One hundred and thirty-five lines resulted in moderate resistance (root score from 2.0-3.0) for *Pythium ultimum* var. *ultimum* and 140 lines for *Pythium ultimum* var. *sporangiiferum*.

6

Efficacy of Metalaxyl and Pyraclostrobin Towards Ohio's Diverse Pythium species and Phytophthora species

Balk, C.*, Wickramasinghe, D., Dorrance, A.E. The Ohio State University, Plant Pathology*

Seedling blights are the third most important disease of soybean. In Ohio, as well as many other Midwest states oomycetes contribute substantially to crop loss every year, primarily through root rot and damping off of seedlings. Better management strategies of oomycetes are needed. Fungicide seed treatments are a proven effective management strategy. Metalaxyl and pyraclostrobin are fungicides currently used as seed treatments to manage *Pythium* species. However, some species are insensitive to one or both of the active ingredients. The objective of this study was to determine the sensitivity of metalaxyl and pyraclostrobin towards a collection of isolates of *Pythium* and *Phytophthora* species in amended agar or amended broth assays. Over 200 isolates of *Phytophthora* spp. and *Pythium* spp. were evaluated for their sensitivity to Metalaxyl at 100 ppm. There was a range of sensitivity towards both fungicides both within and among isolates of the different *Pythium* species. All of the isolates of *Phytophthora* were sensitive to metalaxyl at 100 ppm. These results indicate that combinations of fungicides will be required to manage the diversity of Oomycetes that cause seed and seedling blight of corn and soybean.

7

Nematode Pest Pressure in Long Term Corn Based Cropping Rotations

Bender, B.*, *MacGuidwin, A.E. University of Wisconsin-Madison, Department of Plant Pathology*

Crop rotation mitigates the buildup of many diseases, so data were collected from the 24thand 25thyears of the Wisconsin Integrated Cropping Systems Trial (WICST), to determine the long term impact of crop rotation on the damage potential of nematodes for corn. The WICST is a large scale experiment with all phases of the cropping systems grown every year in a complete randomized block design. Soil samples were collected from the corn phase of the continuous corn, two 3-year organic systems (cash grain and forage) and a pasture control in June 2012 and 2013 and assayed for the nematode community. The Plant Parasitic Index, a metric based on all plant-feeding genera, did not differ among the corn-based cropping systems. Fourteen genera of phytophagous nematodes were detected, with Tylenchus spp., *Helicotylenchus* spp., and *Pratylenchus* spp. the most common (23%, 22%, and 11% respectively). Population densities of the economically important plant parasite, *Pratylenchus* spp., were lower in continuous corn systems compared to the 3-year forage system in both years. The 2012 and 2013 population densities, respectively, were 173 and 150 per 100 cm^3 soil for continuous corn and 380 and 296 per 100 cm3soil for the forage system. The level of nematode disease pressure in the plots is greater now than in baseline assays conducted in the WICST trial 15 years earlier, but 3-year crop rotation did not mitigate the buildup of phytophagous nematode populations.

8

Changes in the Avr1d Locus that Enable *Phytophthora sojae* PR1 Grown on Dilute V8 Agar to Become Virulent on Rps1d

Bloechle, T., *Abeysekara, N., Schmidt, C., Robertson, A., Iowa State University, Plant Pathology*

The pathogenic oomycete, *Phytophthora sojae* causes stem and root rot in soybeans. The pathotypes of various *P. sojae* isolates have been found to change over a relatively short period of time. In a previous rotation study on pathotype shifts in *P. sojae*, microplots planted to different rotations of susceptible and resistant soybeans were inoculated with PR1, a *P. sojae* isolate virulent on Rps7 alone. Over four years, isolates that had gained virulence on Rps1a, Rps1c, Rps1d and Rps3b were recovered from the microplots, independent of selection pressure from the soybean varieties used. Changes in virulence occur as a result of deletion, amino acid changes, and gene silencing at Avr loci. To investigate changes in virulence that may occur during vegetative growth, PR1 was grown on DV8 media and subcultured every week for approximately eight months. Every five weeks, the subculture was pathotyped and genomic DNA extracted. PR1 gained virulence on only Rps1d on two occasions. We will present data showing what changes occurred at the Avr1d locus to enable this gain of virulence and compare these changes to those that occurred in Rps1d-virulent isolates recovered from the rotation study.

9

Thrips Dispersal and Soybean Vein Necrosis Virus (SVNV) in Wisconsin Soybean

Bloomingdale, C.*, Groves, R.L., Smith, D., University of Wisconsin-Madison*

Soybean Vein Necrosis Virus (SVNV) was discovered in the southern U.S. in 2008 and has since been detected throughout the country's major soybean growing regions, including Wisconsin. The only confirmed vector for this Tospovirus is the soybean thrips, *Neohydatothrips variabilis* (Beach). Because

very little is known about SVNV, this study was set up to establish a better understanding of the epidemiology and economic impact of both the virus and thrips vector on soybean. Research objectives include monitoring thrips movements and changes in population composition in Wisconsin, identifying resistant soybean varieties, and determining the impact of the pathogen on yield. To accomplish these objectives, thrips flights were monitored via yellow sticky card traps at multiple soybean variety trials. Total thrips captures were estimated by subsampling from the traps; species identifications are in progress. The first year of the field study revealed two major flights of thrips into Wisconsin soybean fields, the first of which occurred during mid-July and the second between late August and early September. A yield and seed quality study was conducted on a soybean breeding line trial. Regression analysis showed a slight increase in the number of non-developed seeds per pod as disease severity increased. There was little effect of SVNV on yield in this study. A future objective of this project is to find an efficient means of inoculation under laboratory conditions. Laboratory infectivity studies would facilitate investigating how timing of virus infection impacts yield and cultivar resistance screening.

10

Influence of Soil Texture on Yield Benefits for Soybean Cyst Nematode Resistant Varieties.

Broderick, K.,* *Pérez-Hernández, O., Wilson, J., Giesler, L., University of Nebraska-Lincoln*

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). Soil texture is known to affect SCN reproduction and mortality, but its effect on SCN-related yield loss is less understood. The influence of soil texture on yields was investigated by comparing resistant and susceptible cultivars over 7 years at 19 different infested locations (12 irrigated, 7 dry land) throughout Nebraska with initial SCN populations ranging from 300 – 15,000 eggs/100 cc soil. One to four fields were used each year and at least one location in each of the years was considered "sandy" (>50% sand content) with a total of eight sandy fields and 11 non-sandy ("silty") fields. Each location had 3 – 5 resistant and 3 – 5 susceptible varieties replicated 4 times. All resistant varieties in this study had PI 88788 resistance. Susceptible cultivars included high-yielding, popular varieties for Nebraska farmers with a total of 10 susceptible compared to 17 resistant varieties. In sandy fields, yield from resistant varieties was greater than yield from susceptible varieties by an average of 11.4 bu/ac compared to 4.8 bu/ac in silty fields. From 2006 – 2012 the combination of resistance and soil texture more significantly reduced yield loss ($p < 0.0001$) than the use of only resistant varieties ($p = 0.004$). These findings demonstrate that the effect of SCN resistance on yield loss reduction is greater in fields with sandy soil types than in finer textured soil fields.

11

Fusarium thapsinum **from Kansas Sorghum Has Genetic Ties to an African Population**

Bushula, V.,* *Leslie, J., Little, C., Kansas State University*

Sorghum (*Sorghum bicolor*) originates from Africa and was first imported into the USA around 1853. *Fusarium thapsinum* causes both grain mold and stalk rot of sorghum and is found wherever sorghum is grown. We evaluated population structure and genotypic diversity between *F. thapsinum* populations from sorghum in Kansas (167 isolates) and three African countries: Cameroon, Mali and Uganda (81 isolates). Mating type (MAT-1/MAT-2) was determined with PCR. Sexual crosses with *F. thapsinum* tester strains were used to confirm mating type and to evaluate female fertility. Genetic variation and its distribution were determined with amplified fragment length polymorphism (AFLP) markers. Mating

type segregated 101:61 (MAT-1:MAT-2) amongst Kansas isolates and 58:23 amongst African isolates. One strain from Kansas and 10 strains from Africa were female fertile. Based on 52 polymorphic AFLP markers, two subpopulations (subpop1 and subpop2) were inferred using Structure. All 114 members of subpop1 were from Kansas, whereas subpop2 contained 53 Kansas strains and 81 African strains. Genotypic diversity ranged from 67 to 92% in these subpopulations. African strains in subpop2 carried private alleles that were not found in any of the Kansas strains. The subpopulations observed suggest that a portion of the Kansas population of *F. thapsinum* has direct origin in an older population from Africa, with the remainder of the Kansas strains tracing their origin to another population.

12

Evaluation of Hard Red Spring Wheat Cultivar Performance Against Three Major Diseases in North Central North Dakota

Chaparaa, V.*, Eriksmoen, E., NCREC, North Dakota State University, Mint, ND*

Management of a plant disease is well achieved by adopting various cultural practices and fungicide application; however, the use of resistant cultivars is the most effective and economical means of managing several diseases. With an objective of determining the level of disease resistance of widely used commercial cultivars of hard red spring wheat and their yield without any preventative fungicide sprays, a field trial was laid out at three different locations (Minot, Garrison and Rugby) that represent major wheat growing areas in north central North Dakota. 14 spring wheat cultivars were evaluated for the three common diseases in North Dakota that cause serious yield losses; the tan spot (*Pyrenophora tritici-repentis*), leaf rust (*Puccinia triticina*) and head scab (*Fusarium graminearum*). The data on disease incidence and severity was taken for all the three diseases at different stages of the crop. Yields were determined per treatment and later converted to yield per acre. The data collected at each spring wheat location was analyzed as a four-replication randomized complete block design. Least significant difference at the 0.05 probability level (LSD, $p = 0.05$) and coefficients of variation (CV) were calculated from analysis of variance at each location. The LSD is used to compare the performance of two specific cultivars at a time. The results indicated that none of the cultivars had significant disease incidence or severity and the yields obtained were non-significant as well in all the three locations. It has been a fruitful year for spring wheat yields with low disease pressure in north central North Dakota.

13

Assessing the Effectiveness for Nematode Suppression by Radish

Chawner, M.,* *MacGuidwin, A.E., Ruark, M.D., UW-Madison Soil Science*

Oilseed radish (*Raphanus sativus* L) has become a popular cover crop option in the Midwest for the late summer, especially among no-till farmers. Radish has been promoted to reduce compaction, contribute nitrogen (N) to the cropping system, as well as suppress pests. However, little, if any scientific evidence of these benefits exists for radish. The objective of this project is to evaluate the effectiveness of radish in nematode suppression. Radish cover crops were planted in mid-August at two field sites located in Southern and Northeast Wisconsin. Each radish treatment was accompanied by a no cover crop treatment, and all treatments were split for the rate of N fertilizer the following spring after corn was planted. Soil samples were collected for each radish and no radish treatment in plots with no N added as well as plots with 168 kg N/ha added. The samples were collected at specific corn physiological time points throughout the season: planting, 6 weeks after planting, tassel, and harvest. Nematodes were isolated from the soil by wet sieving and centrifugation and identified using a stereomicroscope. Soybean cyst nematodes (*Heterodera glycines*) and root lesion nematodes (*Pratylenchus* spp.) were counted. *H. glycines* was

detected in only a few plots so the data were not analyzed. The root lesion nematode data is currently being analyzed. With this greater breadth of knowledge about radish as a cover crop, farmers in the Midwest will be able to make informed decisions about their cropping systems.

14

Discovering the Next Generation of Late Blight Resistance Genes – Can We Battle *Phytophthora infestans* Evolution?

Chen, Y., *Halterman, D., Department of Horticulture, University of Wisconsin-Madison and USDA-ARS*

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is one of the most destructive plant diseases. RB from *Solanum bulbocastanum* encodes a CC-NB-LRR (CNL) protein that confers partial resistance to most *P. infestans* isolates through its recognition of the corresponding pathogen effector family IPI-O. While the majority of IPI-O proteins are recognized by RB to elicit host resistance (e.g. IPI-O1, IPI-O2), some family members are able to elude detection and block recognition of IPI-O1 (e.g. IPI-O4), leading to inactivation of RB-mediated programmed cell death. Our previous results suggested that in the absence of IPI-O, RB remains in a resting state. A conformational change occurs upon recognition of IPI-O1, which leads to an activated protein. However, when IPI-O4 is present, this effector interacts directly with the CC domain, thus suppressing RB activation. In this present study, RBCC-like fragments from wild potato species (CCwild) were sequenced and tested for intermolecular interactions with IPI-O and other RB CC domains in yeast. Our analysis identified two amino acids within the CC domain that determine CC domain self-association. Hybrid RB constructs (RBblb with the CC domain replaced by CCwild) were made to test IPI-O recognition in *Nicotiana benthamiana*. The goal of this study is to identify naturally occurring or engineered variants of RB that can recognize a broader spectrum of IPI-O effectors or can resist suppression by IPI-O4.

15

Screening Commonly Grown High Tunnel Tomato Varieties for Leaf Mold (*Fulvia fulva*) Resistance

Cleveland, K.M.*, *Jordan, S.A., and Gevens, A.J., University of Wisconsin-Madison Plant Pathology*

Over the last several years in Wisconsin, the construction of high tunnels (HTs) for season extension of locally-grown produce has increased. Tomato is a high value specialty crop and is one of the most commonly grown HT crops. Different environmental conditions prevail in a HT than in an open-field setting requiring unique management. Many recommendations, to date, have been adopted from open-field trials and research from other regions. Tomato varieties commonly grown in Wisconsin HTs have susceptibility to the potentially destructive ascomycete fungus *Fulvia fulva*, which causes leaf mold. This disease can result in defoliation, reducing photosynthetic capacity of plants and leading to a reduction in productivity. Assessment of varietal response to tomato leaf mold is necessary for enhanced, integrated disease management. A 14-day-detached leaf assay was used to assess leaf mold resistance of 12 tomato varieties commonly grown in HTs to an isolate of *F. fulva* collected from Wisconsin in 2013. Varieties evaluated included determinate and indeterminate types, as well as types expressing differing phenotypic characteristics. Results indicated that there were statistical differences between several varieties in their response to *F. fulva* over 14 days. The most susceptible variety was 'Trust' with a Relative Area Under the Disease Progress Curve (RAUDPC) of 0.63. The least susceptible variety was 'Green Zebra' with a RAUDPC of 0.13. Our initial work suggested that it may be possible to enhance management of tomato leaf mold with varietal selection.

16

Poplar as a Model for Dissecting Mycorrhizal Symbiotic Signaling in Woody Perennials

Cope, K.,* *Maeda, J., Venkateshwaran, M., Ané, J.M., University of Wisconsin-Madison*

Mycorrhizal fungi form a symbiotic relationship with their host plant. The two most common types of mycorrhizal fungi include arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi. AM fungi associate with approximately 80% of all terrestrial plant species by penetrating root cortical cells and forming arbuscules. ECM fungi colonize predominately woody plants by forming a fungal mantle that covers the root tip, and a Hartig net of intercellular hyphae that surround epidermal and cortical cells. Both AM and ECM fungi protect the plant from both biotic and abiotic stresses by providing their host plant with macro- and micro-nutrients from the soil; in return, the plant rewards the fungus with carbohydrates. In *Medicago truncatula*, DMI1, DMI2, and DMI3 are three keys genes required for AM symbiosis. Homologs of these genes are present in poplar (Populus x canescens), which associates with both AM and ECM fungi. We hypothesize that these homologs are essential for poplar to associate with AM and ECM fungi. To test this hypothesis, we developed RNAi lines for DMI3, which encodes a calcium-/calmodulin-dependent protein kinase localized to the nucleus. The DMI3-RNAi line and a wild-type line were both subjected to mycorrhization assays to determine if the lines could be colonized by both AM and ECM fungi. Preliminary data indicate that the DMI3-RNAi line could not be colonized by either the ECM fungus *Laccaria bicolor* or the AM fungus *Rhizophagus irregularis* suggesting that this gene is required for both symbioses. These results will be validated by future studies. Furthermore, we will develop additional RNAi lines for homologs of other symbiotic genes, including DMI1 and DMI2. Determining the role of these genes in poplar-AM and -ECM symbioses will allow us to dissect the mycorrhizal signaling pathways in woody perennials.

17

Characterizing the Contribution of Inorganic Nitrogen Species Respiration to *Ralstonia solanacearum* Success in Tomato Xylem

Dalsing, B., *Gonzalez, E., Truchon, A., Allen, C., University of Wisconsin-Madison, Plant Pathology*

For a potentially pathogenic organism to cause disease, the organism must grow in its host. This growth requires a functional electron transport chain, which in turn requires a useable terminal electron acceptor (TEA). In eukaryotes, this means oxygen must be present. Prokaryotes in general have more flexibility, with diverse electron transport chain machinery. Genomic data predict that the bacterial plant pathogen *Ralstonia solanacearum* can use oxygen as a TEA, but can also use four inorganic nitrogen species: nitrate, nitrite, nitric oxide, and nitrous oxide. *R. solanacearum* colonizes host plant xylem, which is low in oxygen. A cytochrome cbb3-type c oxidase mutant, which can't respire in low oxygen, can still grow and cause disease in the plant, suggesting the pathogen can use other TEAs. Transcriptomic analyses revealed that bacterial genes involved in inorganic nitrogen respiration are very highly expressed during growth in host xylem. This suggests that *R. solanacearum* relies on nitrate, nitrite, nitric oxide, and/or nitrous oxide to respire and grow during infection. We investigated the importance of inorganic nitrogen species as alternative TEAs supporting this bacterium's pathogenic success. We used direct measurements to quantify the inorganic nitrogen substrates in tomato xylem fluid and constructed deletion mutants for each enzyme in *R. solanacearum's* denitrifying pathway. We will present data describing the ability of these strains to grow and tolerate denitrification products in culture and in the plant environment. Further, we quantify the effect of these mutations on bacterial wilt virulence.

18

Comparison of Tuber Inoculation Techniques with *Phytophthora infestans*

Dangi, S.*, *Kirk, W., Somohano, P., Michigan State University, Plant, Soil, and Microbial Sciences*

Potato tubers differ in susceptibility to *Phytophthora infestans* and different techniques have been used in various studies to determine the effect of different variables such as temperature, relative humidity, growing season fungicide application and cultivar on the degree of tuber susceptibility. The inoculation techniques were 1) direct injection of a zoospore/sporangial suspension by syringe into the flesh of the tuber; 2) the insertion of colonized agar plugs into wounded tubers; 3) placing inoculum-saturated filter paper onto the eyes of tubers; 4) spraying the tuber surface with a zoospore/sporangial suspension; and 5) direct immersion of tubers into a zoospore/sporangial suspension. The inoculation techniques 1) and 2) involved skin injury of the tubers and the other techniques involved no skin injury. Four genotypes of *P. infestans* (US-8, US-22, US-23 and US-24) and three cultivars of potato (Dark Red Norland, Russet Norkotah and Snowden) were tested. Direct injection of zoospore/sporangial suspension and insertion of colonized agar plugs into tubers caused significantly higher disease incidence and severity compared to all other inoculation techniques. Among the techniques with no skin injury, the immersion method produced consistent disease incidence and severity followed by inoculum-saturated filter paper technique. Spraying the tuber surface with a zoospore/sporangial suspension produced the least infection. Overall, results indicated that the direct injection of zoospore/sporangial suspension and use of colonized agar plugs outperformed all other inoculation techniques; Russet Norkotah was the most susceptible cultivar and US-8 and US-22 were the most aggressive genotypes.

19

Susceptibility of Immature and Mature Potato Tubers to Different Genotypes of *Phytophthora infestans*

Dangi, S.*, *Kirk, W., Somohano, P., Michigan State University, Plant, Soil, and Microbial Sciences*

Potato tuber periderm is a significant morphological barrier that prevents infection by various pathogens. The importance of the tuber periderm to infection by *Phytophthora infestans* was determined. Three different cultivars of potato (Dark Red Norland, Russet Norkotah and Snowden) and four genotypes of *P. infestans* (US-8, US-22, US-23 and US-24) were used in the study. The tubers were immersed in the suspension of inoculum for 24 h to determine the susceptibility of potato tubers at different maturity stages. Periderm resistance to physical injury was determined using a skin set measuring device (Halderson Periderm shear tester). The device measured the amount of torsional force [mNm (milliNewton meters)] required to produce skinning injury. Immature and mature Russet Norkotah required the highest torque (273.5 and 450.7 mNm in 2012 and 298.3 and 398.7 mNm 2013, respectively) in comparison to Dark Red Norland (223.3 and 304.1 mNm in 2012 and 212.0 and 296.1 in 2013 mNm, respectively) and Snowden (208.0 and 316.4 mNm in 2012 and 235.5 and 299.5 mNm) in 2013, respectively. Russet Norkotah had thicker periderm and more phellem cells in the periderm than Dark Red Norland and Snowden. Immature cultivars were most susceptible to infection in both years. Immature Dark Red Norland and Russet Norkotah in 2012 and immature Dark Red Norland in 2013 were most susceptible to infection by *P. infestans*. Genotypes US-22 and US-8 were the most aggressive genotypes of *P. infestans*. These results indicated that the immature potato tubers were more susceptible than mature potato tubers.

20

Investigating Species Composition of the Potato Early Blight Complex in Wisconsin

Ding, S.,* *Cleveland, K.M., Gevens, A.J., University of Wisconsin-Madison, Plant Pathology*

The potato early blight complex is a perennial and destructive foliar fungal disease. Infected leaves develop brown to black necrotic lesions, which negatively impact photosynthesis and eventually cause loss in quality and quantity of tubers. The two causal fungi are debris-borne ascomycetes *Alternaria solani* and *A. alternata*. We currently have a limited understanding of the interactions between the species and the specific role of *A. alternata* in the complex. Our study focused on the population dynamics of both pathogens in a time sequence. Isolates were obtained from symptomatic potato leaves collected from fields during the 2012 growing season in Hancock, Plover, and Grand Marsh, Wisconsin. Identification was based on conidia morphology (from single-conidia-derived cultures) and phylogeny. *A. alternata* (103 isolates in total) tended to appear later and was less frequently isolated than *A. solani* (137 isolates in total). Phylogenetic analysis was conducted based on four genomic regions: ITS, β-tubulin, EF, and Alt1a. *A. alternata* appeared to be more diverse genotypically. For all of the four genomic regions, *A. solani* showed only one genotype, which belonged to the porri species-group; for *A. alternata*, Alta1a resolved four genotype groups, all in the alternata species-group. Since the predominant commercial potato varieties in Wisconsin are susceptible to early blight, the primary management is by fungicide application. Many of the currently used reduced risk fungicides have a high risk for the development of resistance in the pathogen population. It is important to gain an improved understanding of the species composition and overall population response to commonly used fungicides in order to best manage the early blight complex and mitigate fungicide resistance in the short and long term.

21

Biometric Evaluation of Potato Virus Y Foliar Symptoms in Potato

Fulladolsa, A.C.,* *Shay, R., Bornowski, N., Charkowski, A.O., University of Wisconsin-Madison, Plant Pathology*

Potato virus Y (PVY) is a common pathogen of potato that rapidly accumulates in tubers through their vegetative propagation and is mainly managed by seed certification. PVY has caused over 90% of rejections of seed potato lots in the last decade. The emergence and spread of necrotic and recombinant PVY strains causing mild symptoms and tuber necrotic ringspot disease has reduced the efficiency of visual assessments to detect viral incidence and severity. The widespread use of potato cultivars that have transient foliar symptoms or are asymptomatic has also contributed to this problem. To address these issues, we developed a population of diploid plants derived from the asymptomatic tetraploid cultivar Silverton. We used a scale of 0 (no symptoms) to 3 (severe foliar symptoms) to determine disease severity and found that there is symptom expression variation among individuals in the population. Given this variation among infected plants, we needed a more quantitative method of measuring symptoms. Hence, we tested the use of plant anatomical measurements as indicators of disease by comparing infected and non-infected potato plants of three varieties in different locations within a greenhouse. Preliminary results suggest that leaf width varies significantly, but only for some varieties (ANOVA, $\alpha = 0.05$). We can further use the diploid population and the anatomical measurements to study the host genetic factors involved in symptom expression of PVY-infected plants and increase our resources for detecting and managing this important disease.

22

Response of *Fusarium thapsinum* to Sorghum Brown Midrib Lines and to Phenolic Metabolites

Funnell-Harris, D., *Sattler, S., USDA-ARS*

Sorghum lines were bred for reduced lignin for cellulosic bioenergy uses, through the incorporation of brown midrib (bmr) bmr6 and/or 12 into two genetic backgrounds, either as single or double mutant lines. When these lines were assessed for resistance to *F. thapsinum* stalk rot, a cause of lodging, they were as resistant to *F. thapsinum* as near-isogenic wild-type lines. Peduncles of newly identified bmr lines from an ethyl-methanesulfonate-mutagenized population, inoculated with *F. thapsinum*, were as resistant as the wild-type line, BTx623. One bmr line (1107) had significantly smaller mean lesion lengths than BTx623, suggesting a mutation is associated with reduced susceptibility. Growing *F. thapsinum* on medium with ferulic, vanillic, sinapic, syringic and caffeic acids, phenolic compounds derived from the lignin pathway and elevated in different bmr lines, indicated that *F. thapsinum* was tolerant to these compounds. Eight sorghum fungi were also tested for effects on growth by the presence of these compounds and ferulic acid inhibited these fungi. Most of the phenolics inhibited *F. verticillioides* and *F. proliferatum*. Accumulation of phenolic metabolites in bmr plants may inhibit growth of some sorghum pathogens, while other factors, such as aromatic phytoalexins or salicylic acid, may be involved in resistance to *F. thapsinum*.

23

Participatory Evaluation of Potato Varieties on Organic Farms: Opportunities for Education and Research

Genger, R.K., *Groves, R.L., Jansky, S., Rouse, D.I., Charkowski, A.O, University of Wisconsin-Madison, Plant Pathology*

On-farm variety evaluation has the potential to provide farmers with information relevant to their farming environment and methods, enabling better informed variety choices. This farmer-researcher partnership also provides opportunities for researchers to understand the realities of the farm operation, to provide information to farmers on disease and pest issues that impact farm productivity and sustainability, and to design research programs with relevance to on-farm pest and disease problems. From 2010 to 2012, we evaluated commercially available potato varieties on organic farms throughout Wisconsin, and on organically managed land at the West Madison Agricultural Research Station. In 2013, 24 heirloom potato varieties were evaluated, in comparison to the best-performing varieties from previous trials, at 25 organic sites. Varieties were evaluated for early vigor, vine size, insect and disease damage, total and marketable yield, and tuber defects. Tuber defect diseases were a significant cause of loss, with between 24 and 38% culled. Common scab (Streptomyces scabies), silver scurf (*Helminthosporium solani*), and black scurf (*Rhizoctonia solani*) were the most common causes of post-harvest losses. Several heirloom varieties with potential for drought tolerance, early blight resistance, and potato leafhopper resistance were identified.

24

A Comparison of Methods to Detect *Colletotrichum lindemuthianum* in Dry Beans

Halvorson, J.,* *Simons, K., Lamppa, R., Pasche, J., Department of Plant Pathology, North Dakota State University*

Anthracnose, caused by *Colletotrichum lindemuthianum* Sacc. & Magnus, is a serious seedborne fungal pathogen of dry bean (*Phaseolus vulgaris* L.), especially in cool, humid environments. Seed infected with the pathogen has been shown to serve as an important inoculum source for initial plant infection, which can lead to further disease development. In previous research, the level of disease symptoms in the seeds was related to the level of seed to seedling transmission, ranging from 15% in symptomless seeds to 85% in severely diseased seeds. These studies focused primarily on disease development; however, no research has been performed to detail the movement of the pathogen within stem tissue. A previously developed real-time PCR assay was used to quantify seed infection, but was unable to reliably detect the pathogen in symptomless seeds produced in infected pods. In research reported here, greenhouse trials were performed to determine differences in seed to seedling pathogen movement from seeds with infection levels varying from healthy to severe. Data evaluations included seed germination, plant emergence, and fungal quantification comparing growth on solid nutrient media and two real-time PCR assays. In preliminary results, seed germination and plant emergence decreased with increasing seed symptom severity. The newly developed real-time PCR assay for quantification of *C. lindemuthianum* in infected tissue was more sensitive than any other previous methods of pathogen detection. The real-time PCR assay successfully quantified *C. lindemuthianum* in seeds with and without symptoms and seed to seedling transmission of the pathogen within the stem tissue grown from all levels of seed infection. The quantity of pathogen DNA increased as the symptom severity increased. No product was amplified from 17 other *Colletotrichum* species and 12 other fungal and bacterial dry bean pathogens using the new real-time PCR assay.

25

Soybean Seed Treatments Alter Sclerotinia Stem Rot Greenhouse Evaluations

Harbach, C.,* *Koga, L., Hartman, G., Bowen, C., University of Illinois*

A common method used to evaluate soybean genotypes for resistance to *Sclerotinia sclerotiorum* is the cut-stem assay where plants are inoculated under controlled environmental conditions. This method has been used for many years in the Illinois Varietal Information Program for Soybeans (VIPS), which conducts annual disease and pest evaluations of commercial soybean cultivars entered into the University of Illinois Variety Testing trials. The frequency of genotypes with seed treatments entered into the VIPS program has increased in recent years. The objective of this study was to determine if seed treatments affected the evaluation of soybean genotypes in the cut-stem assay by comparing soybean genotypes with and without commercially available seed treatments. Seeds of DSR2400 (partial resistance), IP2991 (intermediate susceptibility), and Resnik (susceptible) were separately treated with Trillex6000 (Bayer), Trillex6000 with Heads-Up (Bayer and HeadsUp Plant Protectants, Inc.), CruiserMaxx (Syngenta) or were untreated. The 12 treatment combinations were blocked in four replications in three experiments, where plants were inoculated at 18, 25, and 32 days after planting (DAP), respectively. At 18 DAP, Trillex6000 with Heads-Up decreased lesion length for all three cultivars compared to Trillex6000, CruiserMaxx, and the control. At 25 DAP, DSR2400 treated seed had reduced stem lesion lengths compared to the control, while Trillex6000 with Heads-Up had reduced stem lesion lengths over all other treatments for IP2991, and for Resnik, Trillex6000 and Trillex6000 with Heads-Up had reduced stem lesion lengths over the control. At 32 DAP, there were no significant differences for DSR2400,

whileTrillex6000 with Heads-Up reduced lesion lengths over CruiserMaxx and the control for IP2991, and Trillex6000 reduced stem lesion length over CruiserMaxx and the control for Resnik. This study showed that seed treatments often reduced lesion lengths in inoculated plants indicating that treated seed may be a factor when evaluating soybeans for resistance to *S. sclerotiorum*.

26

Searching for a Spore Killer: A Meiotic Drive Element in Neurospora Fungi

Harvey, A.,* *Rehard, D., Sharp, K., Kuntz, D., Wilson, R., Brown, D., Shiu, P., Hammond, T., Illinois State University*

Mendelian inheritance predicts that different alleles of the same gene will have an equal chance of being transmitted to the next generation. However, meiotic drive is a phenomenon where certain alleles evolve the ability to bias transmission in their own favor. In this study we are investigating a meiotic drive element called Spore killer discovered in Neurospora fungi over three decades ago. Spore killer in Neurospora is thought to work via a 'killer and resistance' model. This model predicts that there are two tightly linked loci, one for killing and one for resistance. We have recently reported the identification and characterization of the resistance locus. In this work, we have used molecular genetic techniques to identify the killer locus. Knowledge gained from studies of spore killing in Neurospora may help in efforts to develop a Spore killer-based control method for plant-pathogenic fungi.

27

Effect of Volunteer Corn Density on Mycotoxin Production by *Gibberella zeae*

Hollandbeck, G., *Johnson, W., Wise, K., Purdue University*

Gibberella zeae causes Gibberella ear rot in corn and produces mycotoxins such as deoxynivalenol (DON). Volunteer corn is a weed resulting from uncollected kernels from the previous harvest, and is very difficult to control once established, since using a herbicide for volunteer corn control could easily damage the corn crop. Oftentimes genes conferring resistance in volunteer corn are not fully expressed, making it more susceptible to disease. The presence of volunteer corn may also help spread disease, as it provides additional hosts to the pathogen. A field experiment was established in northwestern IN to examine the impact of volunteer corn density on Gibberella ear rot severity and DON levels in hybrid and volunteer corn. The experiment was designed as a split-plot randomized complete block design, with eight replications of each treatment. Two hybrid corn varieties differing in resistance to Gibberella ear rot were planted. Five densities of volunteer corn treatments (0, 0.5, 2, 4, and 8 volunteer plants/m2) were established within each variety. Experimental plots were inoculated with *G. zeae* at silking. At harvest, ten hybrid ears and up to ten volunteer ears were hand harvested from each plot, and the remaining corn was machine harvested. Ears were rated for percent disease severity and tested for DON concentration. Disease levels were low in 2013 due to weather conditions after silking. Yield of both varieties decreased significantly ($P > 0.0001$ for both hybrids) with an increase in volunteer corn density. DON levels were significantly higher in volunteer corn samples of both the susceptible and moderately resistant hybrid ($P=0.0372$; $P=0.0041$, respectively), compared to hybrid corn samples or grain samples taken from combined plot. However, DON levels were very low in the experiment, and the trial will be repeated in 2014 to validate results.

28

Breeding for Resistance to Early Blight in Potato (*Solanum tuberosum* L.)

Jansky, S., *Ma, Y., Halterman, D., USDA-ARS, University of Wisconsin-Horticulture, Madison*

Early blight of potato (*Solanum tuberosum* L.), caused by *Alternaria solani*, is a major cause of economic losses in many potato growing regions. We have identified two early blight resistant clones EB24-24 and EB24-3, which are hybrids between the cultivated (*S. tuberosum*) potato clone US-W4(2x=24) and the wild diploid clone *S. raphanifolium*. These two early blight resistant clones have been crossed as a female to the diploid inbred line M6, which is homozygous for the Sli, which overcomes self-incompatibility in diploid potatoes. Twenty-three plants of EB24-24X M6 and three plants of EB24-3 X M6 were selected for early blight resistance based on a spray inoculation with a sporangial suspension of *A. solani* at a concentration of 20,000 sporangia/ml. Seventeen EB24-24 X M6 plants and one EB24-3X M6 plant were retained because they were resistant to early blight and produced seeds following self-pollination. Additional EB resistance screens were carried out on the EB24-24 X M6 family using both drop inoculation on detached leaves and spray inoculation on whole plants with sporangial suspensions of *A. solani* (20,000 sporangia/ml). One F1 clone from each family was selected and F2 generation for both families planted. These families are being evaluated for seed germination, seedling vigor, fertility, self-compatibility, and early blight resistance. The population with the broadest segregation for early light resistance will be genotyped using the SolCAP Illumina 8303 array. Our goal is to identify molecular markers associated with early blight resistance for use in marker-assisted selection.

29

The Distribution of Soybean Cyst Nematode in Kansas

Jardine, D., *Todd, T., Department of Plant Pathology, Kansas State University*

Economically, soybean cyst nematode (*Heterodera glycines*) (SCN) is the most important soybean plant pathogenic pest in the U.S. SCN was first identified in Kansas in 1985 in Doniphan County. In a survey conducted between 1995 and 1998, the number of known infested counties in the state increased to 34. In 2011 – 2012, a statewide survey was again undertaken. A corn – soybean rotation predominates in Kansas. To allow for this, only fields currently cropped to soybeans were sampled in each of the two seasons. Approximately half the samples were collected after harvest in 2011 and the other half collected similarly in 2012. One sample was collected for each 5,000 acres of soybeans grown in a county. Counties with less than 3,000 acres of soybeans were not sampled. Fields within a county were arbitrarily selected with most samples being collected by county extension agriculture agents. GPS coordinates were recorded for each location. A total of 636 samples were collected. Samples were processed in the KSU Nematology lab by a standard flotation-centrifugation extraction procedure using 100 cm3 of soil. Samples with no detectable levels of SCN were further tested by bioassay. Based on the survey results, 18.9% of Kansas soybean fields are infested with SCN. SCN was detected for the first time in five new counties and brings the total number of known infested counties to 55 (out of 105). Cherokee County had the highest rate of infestation at 95% (19/20) followed by Doniphan County at 75% (9/12). HG type testing of all positive samples is currently under way.

30

Evaluation of Winter Wheat Germplasm for Resistance to Leaf and Stripe Rust

Kertho, A.,* *Francois, M., Ransom, J., Acevedo, M., North Dakota State University*

Wheat leaf rust, caused by *Puccinia tritcina* (Pt), and wheat stripe rust, caused by *P. striiformis* f. sp. *tritici* (Pst), are important foliar diseases of wheat (*Triticum aestivum* L) in the U.S. 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 in the northern Great Plains due to the constant emergence of virulent races of the pathogens. In this greenhouse study, we evaluated 65 winter wheat accessions from regional nurseries in the northern Great Plains (WWR) and 120 from NDSU breeding program (WWNDSU) for seedling resistance to five Pt races; MCDL, MFPS, THBL, TBDG and TBDJ, and one race of Pst; Pstv-37, that are predominant in this region. The Pst race 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. The majority of the winter wheat genotypes were susceptible to the races of Pt and Pst tested. A total of 3 (4.6%) of WWR, 4 (3.3%) of WWNDSU showed resistance to all five Pt races and the one race of Pst. WWR was further evaluated with diagnostic PCR markers to find out the presence of important leaf rust and stripe rust resistance genes. Out of 64 genotypes tested, 13 (20%) had Lr37/Yr17/Sr38, 11 (17.2%) had Lr34/Yr18 and 7 (10.9%) had Lr21. This study shows that many varieties are being grown despite their susceptibility to leaf rust and stripe rust. 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.

31

Using Wild Relatives of Potato to Illustrate Genetic Control Against the Pathogenic Bacteria *Pectobacterium carotovorum*

Lind, J.,* *Charkowski, A.O., University of Wisconsin-Madison, Plant Pathology*

Bacterial soft rot caused by Pectobacterium is one of the most important bacterial diseases of potato and other stored vegetables. Although soft rot resistance is present in wild species, it has not been effectively introduced into cultivated potato due to difficulty of incorporating traits into potato, which is an out-crossing tetraploid. Since potato is grown by vegetative propagation, seed production is also complicated and inefficient compared to other types of vegetables. As a result, little progress has been made in many aspects of potato breeding over the past century. One objective of this research is to characterize soft rot resistance in wild potato species and to generate resistant and susceptible inbred diploid potato lines to aid in characterization of these resistance genes. A second objective is to determine if *Solanum microdontum*, *S. violaceimarmoratum*, and *S. chacoense* accessions that are resistant to *Pectobacterium carotovorum* subsp. *carotovorum* are also resistant to other *Pectobacterium* species such as *P. carotovorum* subsp. *Brasilense, P. atrosepticum* and *P. wasabiae*. The resistance of the inbred lines and a comparison of diploid and inbred diploid potato lines with cultivated tetraploid potato lines will be assessed with plants grown under greenhouse and field conditions. The long-term outcomes of this project are the availability of soft rot resistant potato lines and a contribution to simplifying and sustaining potato breeding and production.

32

The Effect of *Fusarium verticillioides* on *Pratylenchus penetrans* Infection of Corn Seedlings

Lunt, T., *MacGuidwin, A.E., University of Wisconsin-Madison, Plant Pathology*

Pratylenchus penetrans (Pp) and *Fusarium verticillioides* (Fv) are both common pathogens on corn that infect plant roots and mesocotyl upon germination. Fv's effect upon infection by Pp has been mixed. The objective of our study was to determine whether co-inoculation with both Fv and Pp fundamentally changes plant growth characteristics and/or Pp root colonization relative to inoculation with Pp alone. Pioneer P9917XR seed was pregerminated and planted into 500 cm3 pots with autoclaved loamy sand soil inoculated with a sand/cornmeal culture of Fv, fungal growth medium only (control), 2000 Pp or both pathogens in combination. Plants were grown at 27o C in a growth chamber in a randomized complete block design with six replications, with one repeat. After 25 days, plant height, dry shoot and root weights, and root length were determined. Roots and fine root fragments were incubated for the collection of Pp inside roots. Pathogens were recovered from only those plants that received inoculation treatments. Recovered population densities of Pp from corn seminal roots did not differ in plants exposed to Fv compared to plants that were not inoculated with Fv. Recovery of nematodes from adventitious roots was minimal with no significant differences across treatments. No differences in plant morphology were observed between plants inoculated with Pp alone, plants inoculated with Fv alone and plants inoculated with both pathogens concurrently. Experiments are ongoing to determine if different inoculation densities and growth conditions yield contrasting results.

33

Visualizing Stomata as Infection Courts for *Clavibacter michiganensis* subsp. *nebraskensis* on Corn

Mallowa, S.,* *Mbofung, G., Robertson, A., Iowa State*

Clavibacter michiganensis subsp. *nebraskensis* (Cmn) causes Goss's wilt and leaf blight, an economically important disease that has re-emerged throughout the US Corn Belt. Infection is reported to occur through wounds on leaves caused by strong winds and hail; however Goss's leaf blight outbreaks in the absence of obvious injury have been reported. Greenhouse studies have shown the leaf blight can occur without obvious wounding. The goal of this study was to determine if stomata act as infection courts for Cmn and to visualize infection by Cmn. A gfp-labelled strain of Cmn was used to inoculate corn seedlings, at the V5 crop developmental stage in the greenhouse. Epifluorecent and scanning electron microscopy were used to observe Cmn colonization on the leaf surface particularly around stomata. Preliminary studies showed the bacterium around closed stomata 4 hours after inoculation (HAI). Colonization of an epidermal cell in association with a stomate was observed 17 days post inoculation (DPI). Further study is in progress. These data suggest that Cmn can infect corn via the stomata albeit at very low frequency.

34

Evaluation of Genetic Resistance to Common Bacterial Blight in Dry Edible Bean

Maniruzzaman, M.,* *Simons, K., Osorno, J., Pasche, J., North Dakota State University*

Common bacterial blight (CBB) in dry edible bean is caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and found worldwide. CBB can cause up to a 40% yield reduction. Considerable efforts have been made to develop and use molecular markers to select for genotypes resistant to CBB. Two markers commonly used are SU91 and SAP6. Both markers are dominant sequence characterized amplified region

(SCAR) markers linked with a major QTL for CBB resistance and each provide a moderate level of resistance to CBB; however, increased resistance is observed when both genes are present. North Dakota leads the US in dry bean (*Phaseolus vulgaris* L.) production, and produces six market classes, most notably pinto (66%), navy (19%) and black (11%). Neighboring Minnesota, also an important dry bean producing state, produces mainly navy (36%), dark and light red kidney (34%), and pinto (10%) beans. The objective of this study was to screen advanced and preliminary dry bean lines from the NDSU breeding program for the presence of SAP6 and SU91and evaluate their reaction to Xap under greenhouse conditions. To date, 566 plant selections (83 advanced, 465 preliminary and 18 cultivars) have been screened. Among the advanced lines, one navy, three black, one pink, and three small red lines amplified with both markers. Among preliminary lines, seven dark red kidney, two light red kidney, nine pinto, three black, thirteen small red and ten pink lines amplified with both markers. Most notable of these results was the absence of pinto lines in the advanced breeding program with these CBB resistance markers. Genotypic results will be confirmed by phenotyping in the greenhouse. These results will aid in the development of CBB resistant dry bean cultivars with a special emphasis on the needs of North Dakota and Minnesota producers.

35

Evaluating Hop Downy Mildew (*Pseudoperonospora humuli*) Forecasting Tools to Manage Disease in Wisconsin

Marks, M.E., Frost, K.E., Gevens, A.J., University of Wisconsin-Madison, Plant Pathology*

Hops (*Humulus lupulus*) are grown primarily in the Pacific Northwest (PNW) region of the U.S. but there has been great interest in expanding the production of hops in the Upper Midwest U.S. In Wisconsin, approximately 50 acres of hops are in production with expansion to 500 acres likely to occur within the next 2 years. As a result, there is a high demand for information to support disease control. Hop downy mildew (*Pseudoperonospora humuli*) is a potentially devastating disease affecting both foliage and cones throughout the crop life cycle. *P. humuli* can overwinter as mycelia within the hop rhizome and can result in severe infections that lead to total crop loss. A heating degree-day model and a disease risk index were developed in the PNW to predict the spring emergence of shoots that are systemically infected with downy mildew and quantify the overall risk of pathogen infection based on favorable conditions of precipitation, relative humidity, and temperature. The latter tool provides risk thresholds for timing initial and season-long fungicide applications. We applied the downy mildew risk index calculation to Wisconsin weather data from 2008 to 2013 and determined that there was significant annual variation in the number of days that the downy mildew risk index was >500 and in the seasonal occurrence of risk index periods within a given year. Spatially, risk varied greatly across the state of Wisconsin when analyzed by total risk index per year. Such variation by year, within growing season, and geographically suggests the need for site-specific and regular monitoring of weather data for use in effective disease forecasting systems. Our continued efforts will validate and/or optimize both disease models for Wisconsin using historical and current weather and crop data. And, we are working to build web-based tools to ease grower access to site-specific disease forecasting.

36

Differential Effects of Temperature and Selected Phytochemicals on Development and Infectivity of Two Economically Important Plant Parasites, the Soybean Cyst Nematode, *Heterodera glycines*, and Root-Knot Nematode, *Meloidogyne incognita*

Masler, E.P., *Rogers, S., Chitwood, D., US Department of Agriculture*

Plant-parasitic nematode control relies on a number of approaches, including generation of resistant cultivars and limited application of chemicals. The latter are under severe restrictions and removal from use. Production of resistant cultivars is challenging, and effectiveness is undermined by changing pest populations. Consequently, much research is now focused on discovery of control methods that are environmentally responsible, effective and adaptable to varying target nematodes. At the core of such efforts is an understanding of plant-parasitic nematode biology and the response of these parasites to environmental challenges. Of particular interest are metabolic and developmental mechanisms that offer novel processes for control. Using in vitro approaches, we temporarily exposed developing embryos (eggs) of *H. glycines* and *M. incognita* to low temperature (5oC) or to phytochemicals, isothiocyanate or a catechin polyphenol, as mimics of environmental challenges. After treatment removal, embryonic development, hatching, and infectivity were monitored. Exposure of eggs to low temperature decreased hatch by 50 % ($P < 0.05$) in each species. The decrease was associated with developmental arrest initiated during early embryonic stages but apparent only in more advanced embryos. In contrast to temperature, hatch suppression by the polyphenol epigallocatechin gallate (EGCG) was significantly greater ($P < 0.05$) in *M. incognita* (69%) than in *H. glycines* (23%). The differential responses suggest that specific metabolic pathways were affected, in contrast to a general metabolic depression at low temperature. Embryogenesis was not affected, but EGCG inhibition of protease and chitinase may be involved. Treatment of infective larval stages with benzyl isothiocyanate (BITC) had behavioral effects and reduced infectivity ($P < .05$) on soybean 97% by *M. incognita* and 80% by *H. glycines*. Details of the developmental systems affected by the treatments, and the use of environmental cues to prospect for endogenous nematode molecules as novel control leads are presented.

37

Characterization of *Clavibacter michiganensis* subsp. *nebraskensis* Isolates in Minnesota

McNally, R.R., *Curland, R., Hoerth, S., Ishimaru, C., Malvick, D., University of Minnesota*

The Gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn) is the causative agent of Goss's wilt, an agronomically important disease of corn. The occurrence and distribution of Goss's wilt in North America has increased dramatically in the last decade and, as such, Goss's wilt is currently considered a re-emerging disease threat. In 2009 Goss's wilt was confirmed for the first time in Minnesota. Although Goss's wilt epidemics can reduce corn yields up to 50%, information regarding the ecology, epidemiology and virulence of Cmn is sparse. In response, 128 isolates of Cmn were screened in greenhouse assays against multiple corn hybrids to evaluate the virulence potential of Cmn populations in Minnesota. Cmn isolates exhibited different levels of virulence. In susceptible corn hybrids, some Cmn isolates are highly virulent while others appear non-pathogenic. The existence of non-pathogenic Cmn isolates is in accordance with observations of other *Clavibacter* subspecies and calls into question the role of non-pathogenic *Clavibacter* populations during epidemic development. To study this phenomenon, the identity of Cmn isolates was confirmed using immunological and polymerase chain reaction (PCR)-based techniques. Cmn isolates were inoculated into *Nicotiana tabacum* to test for the ability to elicit a hypersensitive response in a non-host species. The distribution of potential virulence factors was also explored in multiple Cmn isolates via PCR amplification. In addition, to study interactions *in planta*, co-

inoculations of susceptible corn hybrids were conducted using varying concentrations of virulent Cmn isolates and Cmn isolates exhibiting less virulence. Understanding interactions among Cmn isolates and the factors that distinguish pathogenic and non-pathogenic isolates will be important components of understanding virulence in Cmn as well as the epidemiology of Goss's wilt.

38

Effect of Different Corn Residue Levels on Goss's Wilt of Corn

Mehl, K.,* *Ames, K., Bradley, C.A., University of Illinois*

Goss's wilt of corn, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn) is a reemerging disease in Illinois. A shift towards corn production practices that utilize short to no rotations and conservation tillage practices has increased the risk of Goss's wilt epidemics in Illinois. To better understand the role of corn residue on Goss's wilt epidemics, a field study was conducted in 2012 and 2013 near Urbana, IL. Different tillage practices (no-till, chisel plow, and moldboard plow) were utilized to develop different levels of corn residue in fields that were inoculated with Cmn the prior year. Goss's wilt incidence and severity data were collected and a disease severity index (DSI) was calculated for each plot, and plots were harvested and yields were determined. Plots with the least amount of corn residue (chisel plow and moldboard plow) had significantly lower DSI values than plots with greater amounts of corn residue (no-till). No significant differences in yields across tillage treatments were observed in 2012, but in 2013, the highest yields were observed from plots that had the lowest amounts of corn residue (moldboard plow). These results suggest that in situations where conservation tillage practices are used in corn-intensive rotations, the risk of Goss's wilt is high, and corn growers should place a priority on planting hybrids with high levels of Goss's wilt resistance under these situations.

39

Report of Recovery of Soybean from Sudden Death Syndrome Caused by *Fusarium virguliforme*

Navi, S., *Jing, L., Yang, X., Iowa State University*

Sudden death syndrome (SDS) of soybean (Glycine max) caused by *Fusarium virguliforme* (FV) is an economically important disease that causes major yield losses to soybean producers. Characteristic symptoms of this disease are interveinal chlorosis and necrosis of leaves and premature defoliation. Soybean plants showed recovering from SDS infection by producing healthy plants in several seed treatment field trials since 2011. This phenomenon of healthy regrowth (recovery) from SDS infected plants in soybean seems to be a new type of resistance. The term recovery is the production of symptomless leaves in a whole plant or plant branch that previously had one or more uni- or trifoliate leaves with SDS symptoms. Experiment was set up in RCBD with four replications each with five cups. Seeds of four commercially untreated Pioneer varieties (P92M40RR, P92M76RR, P92Y51RR, and P93Y13RR) were treated with biocontrol agent (BCA) to test against SDS in the greenhouse. Treated seed were planted in 8 oz cups containing sterilized potting mixture (2 parts sand 1 part soil) and 1%FV (grown on steam sterilized white milo), equal number of untreated seed as control. Varieties tested during March-April 2012 (as above) and April-May 2014 (P92Y60RR) showed substantial variability of recovery. Significant differences ($P<0.05$) of recovery observed in BCA treated compared with untreated. Mean recovery of SDS infected plants across varieties was 4.16% (range 3.22% to 6.07%) in BCA treated/herbicide sprayed and 5.84% (range 5% to 6.19%) in BCA treated/herbicide not sprayed treatments. While, in untreated controls the values were 2.41% (range 0%-4.33%) in herbicide sprayed and 3.62% (range 2.23%-4.66%) in unsprayed treatments. It may be significant to determine if such recovery is similar to reports in pearl millet and sorghum downy mildews. Soybean is self-pollinating;

development for higher yields coupled with recovery phenomenon could likely be more durable than major gene resistance to SDS.

40

Optimizing Fungicide Application Timing to Control Fusarium Head Blight in Winter Wheat

Noveroske, A.,* *Wise, K., Purdue University*

Fusarium Head Blight (FHB) of wheat, caused by the fungus *Fusarium graminearum*, is currently considered one of the most economically important diseases in the North Central United States. The fungus causes light-weight "tombstone" grains to form and produces the mycotoxin deoxynivalenol (DON), reducing the yield and quality of the grain. Currently, farmers rely heavily on the Sterol Demethylase Inhibitor (DMI) triazole fungicide Prosaro (Bayer CropScience) to protect their crop. The optimum fungicide application timing is early anthesis; however, environmental conditions at this growth stage can inhibit fungicide application. Field trials were conducted in the 2012-2013 growing season to determine the impact of post-anthesis fungicide timing, in conjunction with initial infection by *F. graminearum* on FHB and DON. The experiment was established at the Agronomy Center for Research and Education in West Lafayette, IN. The experiment was a random complete block design with four replicates. Treatments consisted of single applications of Prosaro at 475 mL/ha applied at Feekes Growth Stage 10.5.1 (anthesis), and anthesis + 1, 3, 5, 7, 9, and 11 days. All plots were inoculated with macroconidia of *F. graminearum*. Non-treated inoculated plots served as controls. Disease index was assessed eight days after the final treatment. DON and yield were evaluated post-harvest. Prosaro significantly reduced levels of FHB index across treatments ($P = 0.0145$) with the greatest percent disease control achieved with fungicide application 11 days after anthesis (58%). Prosaro application at all timings also significantly reduced DON ($P < 0.0001$) compared to the non-treated control. However, the lowest DON level observed, 3.5ppm, was still higher than the 2.0 ppm recommended for sale of grain. Results indicate that post-anthesis fungicide applications may be useful at reducing FHB and DON when conditions are favorable for disease development.

41

Evaluation of Inoculation Methods for *Fusarium avenaceum* in Field Peas

Odom, J.,* *McPhee, K., Porter, L., Pasche, J., Department of Plant Pathology, North Dakota State University*

Fusarium solani f. sp. *pisi* and *F. avenaceum* are reported to be the most important species in the Fusarium root rot disease complex in major pea production areas in North America. The effective evaluation of host plant resistance to *Fusarium* spp. under greenhouse conditions is invaluable in accelerating the development of disease resistant cultivars. However, the development or identification of screening methods that correspond to field evaluations can be particularly challenging with a root rotting pathogen complex. To date, no research to validate the various greenhouse inoculation methods used for Fusarium root rot in peas has been performed. Lines and cultivars with resistance to *F. solani* f. sp. *pisi* have been identified; however, to our knowledge, no sources of resistance to *F. avenaceum* have been reported. The goal of the research reported here is to provide researchers and breeders with tools to accelerate the development of Fusarium root rot-resistant pea cultivars. Three isolates of *F. avenaceum* were used to inoculate ten dry pea cultivars using three inoculation methods under greenhouse conditions. Preliminary results indicate differences in root rot severity across cultivars, among isolates within *F. avenaceum*, and across methods used. Some level of resistance, depending on method, was observed in the Austrian Winter pea types. However, these have unfavorable quality characteristics, making it

difficult for breeders to incorporate traits from these into dry green and yellow pea types. Severe root rot was observed on some cultivars, but levels differed based on *F. avenaceum* isolate and method. These results further illustrate the complexities in accurately identifying resistance to root rot pathogens.

42

Sensitivity of Kansas *Sclerotinia homoeocarpa* Isolates to DMI Fungicides

Ostrander, J.,* *Kennelly, M., Kansas State University*

Dollar spot, caused by *Sclerotinia homoeocarpa*, is a serious disease of intensively managed turfgrass such as creeping bentgrass (*Agrostis stolonifera*) in golf course fairways and putting greens, and fungicides are commonly used to maintain turfgrass at acceptable quality. *S. homoeocarpa* has shown resistance to sterol demethylation inhibitor (DMI) fungicides in many locations, but isolates in Kansas have not been tested. Our objectives were: (1) to determine the sensitivity of 74 Kansas *S. homoeocarpa* isolates to the DMI fungicides propiconazole, metconazole, tebuconazole, and triticonazole using *in vitro* mycelial growth assays; (2) to determine a single discriminatory concentration for each fungicide to be used in future testing; and (3) to examine the correlations among sensitivity to the different fungicides. Isolates were collected from 12 sites in Kansas, primarily golf course putting greens. In vitro growth assays were conducted at concentrations of 0, 0.001, 0.01, 0.05, 0.1 or 1.0 µg a.i. (active ingredient)/ml, and colony diameters were measured. For each fungicide concentration, the percent relative growth for each isolate was calculated by comparing the growth on amended media (GOA) compared to the growth on the non-amended control (GOC) by the equation (GOA/GOC) x 100. Using the log10 values of the fungicide concentration, regression was used to calculate the concentration to reduce growth by 50% (log10EC50). For all four fungicides, the log10EC50 values formed a unimodal curve but were not normally distributed. The mean EC50 values were 0.0163, 0.038, 0.0612, and 0.0994 µg a.i./ml for metconazole, propiconazole, tebuconazole, and triticonazole, respectively. Correlations were significant and positive for all pairwise comparisons of log10EC50 values. Regressions using discriminatory concentrations tested were significant. The most predictive concentrations were 0.01, 0.05, 0.05, and 0.10 µg a.i./ml for metconazole, propiconazole, tebuconazole, and triticonazole, respectively. This study provides a baseline for further monitoring and streamlining of resistance assays.

43

Population Dynamics of *Heterodera glycines* in Corn-Soybean Rotated Fields in Nebraska

Pérez-Hernández, O., *Broderick, K., Giesler, L., University of Lincoln-Nebraska*

Crop rotation and use of resistant varieties are the major practices to manage the soybean cyst nematode (SCN, *Heterodera glycines*) in the north central region of the U.S. In Nebraska, where a large soybean area is grown under irrigation, little is known on the long-term dynamics of SCN in corn-soybean rotated production fields. The objective of this study was to characterize the population dynamics of *Heterodera glycines* in commercial fields annually rotated with corn and soybean in Nebraska. Ten fields with a history of SCN and in an annual rotation regime were randomly selected in 2009 and the population dynamics of SCN in each field was monitored until 2012 through spring sampling. In each field, ten 3 x 3-m sampling grids were selected systematically using GPS methodology and SCN population densities were determined with standard SCN laboratory procedures. The SCN population densities among fields were compared considering the soybean resistance source planted and the covariance structure of the sampling grids within each field. SCN population densities declined after the first corn rotation year, with an increased decline in fields with initial population densities ≥1,500 SCN eggs/cm^3 of soil. The results of

this study suggest that in Nebraska, once SCN population densities decline to a level ≤1,000 eggs/cm^3 the effect of either corn rotation or SCN resistant soybean varieties could become less apparent.

44

Investigating Spore Killer of *Fusarium verticillioides*

Pyle, J., *Patel, T., McCall, M., Proctor, R., Brown, D., Hammond, T., Illinois State University*

Maize is one of the most important crops in the world. *Fusarium verticillioides* may colonize maize as an endophyte or as a pathogen, causing disease at any life stage of the plant. During growth on maize, *F. verticillioides* can synthesis a number of mycotoxins including fumonisins, which have been linked with human esophageal cancer and birth defects. To control fumonisins, we are exploring the use of a selfish genetic element known as Spore killer. For example, we envision creating a biocontrol strain that can harness the genetic transmission-distorting properties of Spore killer to modify the genetic structure of agricultural populations of fungi. In the case of *F. verticillioides*, this could allow us to target fumonisin synthesis in an agricultural population and limit the contamination of agricultural products. Here we report our progress towards the necessary first step of cloning and characterizing the locus that causes spore killing in *F. verticillioides*.

45

Host Translation Factor Requirements of the Triticum Mosaic Virus

Roberts, R.*, *Zhang, J., Mayberry, L., Browning, K., Rakotondrafara, A., University of Wisconsin-Madison, Plant Pathology*

To promote fitness, cells regulate synthesis of proteins to modulate levels in response to external cues. Included are the initiation factors required for translation of most eukaryotic mRNAs. Initiation requires a 5'cap to recruit the cap-binding factor eIF4E and the scaffold protein eIF4G. Instead of a 5'cap, many RNA viruses contain an internal ribosome entry site (IRES) that recruits ribosomes to the mRNA in a 5'- and cap-independent manner. Recently, we showed that Triticum Mosaic Virus (TriMV, Potyviridae) contains an IRES in its 739-nucleotide 5'UTR. Compared to other potyviruses, the TriMV IRES is highly structured, requires longer sequence, and translates more efficiently. This behavior questions whether this IRES has different factor requirements than known plant virus IRESes, which we aim to investigate. We tested the translation ability of the TriMV 5'UTR in vitro under limited cap-binding conditions by adding exogenous m7GTP cap-analog to the translation reaction. Under these conditions, TriMV 5'UTR-mediated translation was largely unaffected compared to a capped mRNA control. Adding recombinant eIF4E provided no advantage to IRES-mediated translation. We then competed free TriMV 5'UTR RNA against a capped mRNA in translation, and demonstrated that the 5'UTR is able to inhibit translation of the capped RNA. Adding eIF4F was sufficient to recover this loss to near 100%, while the addition of eIF(iso)4F recovered translation to about 50%. This suggested that the TriMV 5'UTR sequesters eIF4F. To reveal factor dependency of TriMV IRES-mediated translation, we reconstituted translation in wheat germ extract depleted of the cap-binding complexes. Our preliminary data suggests that eIF4F, not eIF(iso)4F, is able to restore TriMV IRES-dependent translation. Together, our data reveal that the TriMV 5'UTR contains an IRES that requires eIF4F for its translation. Our long-term goal is to identify trans-acting factors that enhance viral translation, which could have major implications in breeding for resistance.

46

Development of Conventional PCR Assays for the Diagnosis of *Stenocarpella* spp.

Romero Luna, M.*, *Aime, M., Woloshuk, C., Wise, K. Purdue University*

Diplodia ear rot occurs almost everywhere corn is produced. The causal agents of this disease are two species of the *Stenocarpella* genus, *S. macrospora* and *S. maydis*. In addition to ears, both pathogens can also infect leaves and stalks, and both are present in the North Central region. It is nearly impossible to visually distinguish between the two pathogens based on plant symptoms and fungal signs. The diseases can be distinguished based on conidia morphology, but this technique can be time consuming and expensive. To facilitate accurate and rapid pathogen identification, specific primers of 18-20 nucleotides in length for each specie were designed, targeting a portion of the ITS region of the fungal genome. A conventional PCR method was developed using these primers, and both sets of primers successfully amplified fragments from the specific target pathogen. To evaluate the reproducibility of these primers, 70 *S. maydis* and 11 *S. macrospora* were screened. A single 1.7 Kb and 800 bp fragment were successfully amplified for each *S. maydis* and *S. macrospora* isolate, respectively. To test specificity, seven additional fungal corn pathogens were tested, including *Fusarium verticillioides*, *Aspergillus flavus*, and *Exserohilum turcicum*, and no significant cross-reaction with non-target organisms was observed for either of the two assays. Development of real-time PCR primers are underway.

47

Mapping Soil bacterial Diversity in Michigan Potato Production Systems Using GIS Coupled with Parallel Sequencing

Rosenzweig, N*., Steere, L., Santa Maria, A., Steinke, K., Kirk, W., Michigan State University, Plant, Soil, and Microbial Sciences*

Potato production in Michigan (MI) is valued at $128 million and ranks seventh nationally. A grower survey identified that an increase in soil-borne disease complexes and declining yields in parts of MI have raised concerns of inability to serve the high-demand chipping market. Growers in MI identified that soil microbial activity and diversity are important factors related to soil health and limiting yield. Additionally the amount of acreage affected by potato common scab (PCS) caused by *Streptomyces* spp. increased by 11% or more over last decade. PCS continues to be an annual concern for commercial potato production in MI, but the soil ecology related to PCS is not adequately understood. Twenty soil samples from 26 fields (n=520) scheduled to be in potato production were taken and GPS marked in the fall/spring of 2012-13, and total genomic DNA was extracted. Next generation sequencing targeting the 16S rRNA gene, and dual indexing allowed high throughput processing of samples simultaneously. The total number of sequences identified to phyla, class, order, family and genus was 28, 81, 140, 300 and 814 respectively. Sequencing results and information gathered on yield and scab pressure from each point was used to generate multi-layer GIS-based maps. The results of this study allowed the mapping of bacterial diversity and richness across potato production fields in relation to soil physical properties, scab disease severity and total yield.

48

Identification of Host-Microbe Interaction Factors in the Soft Rot Pathogen, *Pectobacterium carotovorum*, Using Supervised Machine Learning

Rubio, I.*Ma, B., Charkowski, A.O., Perna, N., University of Wisconsin-Madison, Plant Pathology*

Host-microbe interactions are complex biological processes that involve a substantial number of genes from each organism. Genome sequencing provides many insights into the genes involved, but traditional bioinformatics approaches struggle with unknown genes and have high false positive rates, discouraging experimental verification. A supervised machine learning strategy was developed to overcome these limitations and generate a list of novel candidate genes involved with host-microbe interactions. To test this new approach, we selected the soft rot pathogen, *Pectobacterium carotovorum*, due to its worldwide importance, large amount of genomic data available, and tractability in laboratory environments. We constructed a mini-Tn5 transposon mutation library in *P. carotovorum* and will use a PCR-based strategy to identify strains with mutations in genes of interest. We are focusing on genes of unknown function that are predicted to either be important for plant-microbe interactions or not involved in them. The mutants will be tested with multiple plant assays to determine if they are impaired in survival, growth, persistence or disease development in potato or other plant hosts. Our long-term goal is to develop a generalized machine learning tool to aid in identification of microbial genes involved in complex traits. This tool would be particularly useful with host-microbe interactions that are difficult to manipulate through standard mutagenesis methods.

49

Identification of Sources of Resistance to Wheat Bacterial Leaf Steak in Triticale

Sapkota, S.,* *Liu, Z., Mergoum, M., North Dakota State University*

Bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (XTU), is a common bacterial disease of wheat (*Triticum aestivum* L.) and other small grains including triticale (*Xtriticosecale* L.). Recently, the disease has become increasingly important in the Northern Great Plains of the United States where the majority of the US hard red spring and durum wheat are produced. BLS is capable of causing a significant reduction both in grain yield and quality. Management of BLS is challenging because no chemical method has been found effective in the field. Utilization of genetic resistance is the only option to manage the disease. However, breeding for BLS resistant cultivars is difficult due to the lack of reliable resistance sources. Using a recently established protocol for BLS evaluation in the greenhouse, we have evaluated 254 triticale lines to BLS. A wide range of reactions from highly resistant (disease score = 1) to highly susceptible (disease score = 5) was observed. The resistant reaction was characterized by the development of weak chlorosis or water-soaking, which has not been observed among wheat lines. Bacterial counting indicated that the bacterial growth within plant tissue was correlated well with the level of BLS susceptibility. A total of 17 lines were identified with the high levels of resistance, including a few which have been reported previously. These identified resistant lines could serve as a good source of resistance for wheat breeding programs.

50

Inoculum Substrate Influences Root Development and Causes Discoloration Similar to *Fusarium solani* Infection of Soybean

Sessoms, F., *Otto-Hanson, L., Barbeau, A., Malvick, D., Kurle, J., Plant Pathology, College of Food, Agriculture, and Natural Sciences, University of Minnesota*

Whole grain such as sorghum seed infested with a pathogenic fungus is frequently used as inoculum in resistance screening trials. In previous experiments we observed that uninfested substrates, particularly sorghum seed, applied as controls caused lesion-like symptoms and impaired plant development. Our objectives were to determine if 1) *Fusarium solani* infested seed and uninfested seed differed in their effect on germination, root development, and lesion-like root discoloration and 2) if soybean cultivars differed in their sensitivity to the effect of the substrates. In experiments conducted in different growth media and Phytagel, uninfested red sorghum, white sorghum, barley, and oats caused lesion-like root discoloration. In growth media experiments, the root biomass was reduced by uninfested red sorghum when compared to the soil only control. In addition, tap root length and lateral root number were reduced by red sorghum in Phytagel experiments. However the lesion-like discoloration differed from lesions caused by *Fusarium solani* infection. Soybean cultivars differed in their sensitivity to both uninfested and infested substrates and the responses of individual cultivars were correlated. The spurious effects, possibly caused by allelopathic compounds leached from the uninfested seed, could confound results of resistance screening evaluations.

51

Crossing Path(ogen)s: *Aspergillus flavus* Chlamydospore Development is Induced by Exposure to a *Ralstonia solanacearum* Lipopeptide

Spraker, J.,* *Sanchez, L., Dorrestein, P., Keller, N., University of Wisconsin-Madison*

Fungi and bacteria are two of the primary pathogens of plants and they often have overlapping host-ranges, however shockingly little is known of how pathogens in similar environments interact, be it in host or outside of plant hosts. In examining the interaction between two economically important pathogens of peanut, *Aspergillus flavus* and *Ralstonia solanacearum*, a fungus and bacterium, respectively. We histologically characterized the formation of chlamydospores, a new morphological trait in *A. flavus*. These structures are formed in response to diffusible bacterial compounds as indicated in cell-free assays. This research is focused on characterizing the chemical and genetic dynamics of the interaction that results in the formation of chlamydospores. Matrix-assisted laser desorption time-of-flight (MALDI-TOF) Imaging Mass-Spectrometry (IMS) is being utilized to discover what compounds are involved in this diffusible inter-Kingdom communication. Targeted bacterial gene disruptions and bioassay were utilized to identify bacterial genes, encoding a hybrid nonribosomal peptide/polyketides synthase, responsible for the formation of these compounds. Subsequent high-resolution tandem mass-spectrometry (HR MS-MS) and NMR experimentation has facilitated compound characterization. These data demonstrate that inter-Kingdom chemical communication between pathogens can impact morphological transitions, which may play an important role in rhizosphere biology.

52

Characterization of Pythium spp. Pathogenicity and Virulence on Common Bean (*Phaseolus vulgaris* L.)

Toporek, S., *Rojas, J., Hanson, L., Chilvers, M., Michigan State University*

Common bean (*Phaseolus vulgaris* L.) is a globally cultivated and important legume food crop. Commercial bean production in the U.S. is largely restricted to the northern states, where cool, spring planting temperatures can favor Pythium root rot disease. Early infection from *Pythium* spp. can cause both pre- and post-emergence damping off, potentially causing significant yield loss. *Pythium* spp. many of which having a broad host range, are often ubiquitous in soil and can survive through the winter as thick-walled oospores or chlamydospores. A high throughput screening method was developed in order to screen 69 Pythium and 15 other oomycete species for their pathogenicity and virulence on common bean. Many of these species have never been correlated to root rot of common bean. The screening was performed at two temperatures, 20°C and 26°C, because of the variability that exists between optimum disease-causing temperature among species. The root-rot susceptible red kidney variety 'Red Hawk' was challenged using an inoculum layer method. Root and shoot dry weights were recorded to determine the impact of the tested species on plant growth of 10-day-old seedlings.

53

Development of R-Based Software For Analysis of Spiral Gradient Endpoint

Torres-Londono, G.,* *Hausbeck, M.K., Michigan State University, Plant, Soil, Microbial Sciences, and Hao, J., University of Maine*

Spiral autoplate technique has been commonly used to determine the sensitivity of many microorganisms to various antimicrobial compounds. The effective concentration (EC) of any chemical using the spiral plate technique is usually calculated using associated software, such as the Spiral Gradient Endpoint (SGE). To establish a free and easy-to-use platform, an R-based statistical package (EC50) was developed to calculate the EC of compounds. Final concentrations, at different dilution points along the plate, of 13 antimicrobial compounds with molecular weight (MW) ranging from 171 to 823 g/mole and one virtual compound with MW = 1,000 g/mole, were calculated at various concentrations, time and agar height values combinations using SGE. The model calculates the concentration of the product at 20-mm radial distance from the center of the plate based on the product's MW, and other user-defined parameters, including radial distance from the center of the plate. The software can calculate individual or multiple maximum inhibitory concentrations (TER), minimum inhibitory concentrations (ER) or EC. In addition to the R packages, a web-based graphic environment Shiny package extension (EC50) was developed to provide a graphical user interface. Graphical and statistical comparisons between SGE software and the EC50 package demonstrated high similarity between the methods; the paired t-test and Chi-square analysis presented values of 0.994 and 1.0, respectively, at 95% of significance. Different from SGE that is a Windows based software, the EC50 package was successfully tested on Windows, Linux and Mac operating systems. During a processing speed test, EC values for a 12,000 data points were calculated at 136 seconds using Ubuntu 12 (64 bits). Therefore, the EC50 package is a reliable, cost-free and expeditious program that can be used as an alternative to commercial software.

54

From Drupe to Seedling: Seed Pathology of American Ginseng

Townes, A.,* *Hausbeck, M.K., Michigan State University, Plant, Soil, Microbial Sciences*

American ginseng (*Panax quinquefolius*) is a medicinal perennial herb valued in the U.S. and Asia for its dried root. In North America, the largest acreage of commercially cultivated ginseng is in Ontario, Canada, and Wisconsin. Other states, including Michigan, also grow the crop. A lengthy seed stratification period and multiple causal agents that incite damping-off create challenges for growers who typically harvest their own seed for future plantings. Plant pathogens may originate on and/or in drupes and proliferate during stratification. The objective of this study was to identify pathogens associated with ginseng seed during the various stages of development and stratification. Thirty samples each of drupes, green (non-stratified) seed and stratified seed were obtained from a grower cooperator and the tissue plated onto water agar and dichloran rose bengal choloramphenicol agar. The seeds were dissected so that seed coat and endosperm were plated separately. Mycelia were transferred to potato dextrose agar for identification based on morphological characteristics. The following were found on all seed stages: *Alternaria alternata*, *A. panax*, *Fusarium* spp., *Penicillium* spp. and *Mucor* spp. A high incidence of *A. panax* (44%) and *A. alternata* (80%) were associated with drupes; *Fusarium oxysporum* (13%), and Mucor spp. (31%) were also detected. Although the seed coat of green seed yielded a high incidence of A. panax (27%) and *A. alternata* (67%), the endosperms had a much lower incidence (7%). *Pythium* spp. (40%) were associated only with stratified seed which also had the highest incidence of *Fusarium* spp. (F. oxysporum, 33%; *F. solani*, 33%; and *F. avenaceum*, 13%). All may cause damping off or foliar disease on ginseng or cause a seed rot in storage on other crops. These results demonstrate the need for ginseng seed treatments that protect seedlings from pathogens and can be applied without adverse effects on germination.

55

Bacterial Nucleases as Novel Virulence Factors of *Ralstonia solanacearum*

Tran, T.,* *Hawes, M., Allen, C., University of Wisconsin-Madison, Plant Pathology*

Bacterial nucleases are well-known for their role in recycling genetic material for nutritional purposes. Recently, extracellular nuclease was identified as an important virulence factor in a number of human pathogens. Bacterial DNases contribute to the degradation of the DNA backbone of neutrophil extracellular traps (NETs) and promote the dispersion of the pathogen from the point of infection and also release from the traps harmful compounds that trigger leukocyte cell-death. We found that in response to *Ralstonia solanacearum*, the plant root border cells produce DNA-containing structures resembling NETs to capture bacterial cells. Interestingly, *R. solanacearum* also possesses two predicted secreted nucleases that are expressed during pathogenesis, namely NucA and NucB. A mutant of both genes had reduced virulence on a susceptible tomato cultivar when being introduced by either root inoculation or stem injection. This strain also caused less stunting on roots of tomato and pea seedlings. Interestingly, deletion of these nucleases caused the bacterium to be more contained from the point of inoculation in tomato stem. Preliminary results also suggested that these nucleases, beside their possible role in degrading plant extracellular trap, may be important for nutritional purpose and modulation of bacterial biofilm.

56

Deletion, Complementation, and Characterization of narXL in *Ralstonia solanacearum* GMI1000

Truchon, A., *Dalsing, B., Allen, C., University of Wisconsin-Madison*

The plant xylem is a low oxygen, low nutrient environment containing a high level of inorganic nitrogen species, such as NO3-. Many organisms are incapable of living in such environments, perhaps due to the lack of oxygen, as most organisms rely on an aerobic respiratory chain using oxygen as a Terminal Electron Acceptor (TEA). During low oxygen conditions some bacteria switch from aerobic to anaerobic respiration and use nitrogen species as TEAs in the process of denitrification. One such component of this metabolic change is the two-component sensor-regulator NarXL. NarXL senses nitrate and controls denitrification-regulated gene expression. As *R. solanacearum* enters the plant xylem we suspect that it encounters low oxygen conditions and implements denitrification for anaerobic respiration. Using a narXL minus mutant, we will perform in planta and in culture tests to confirm narXL's role in xylem growth success. We expect the narXL mutant strain will have reduced ability to grow anaerobically, but will grow normally under aerobic conditions in culture. This may also lead to a reduced ability to cause disease *in planta*. Analyzing the impact of NarXL on *R. solanacearum* growth during infection will increase understanding of the overall metabolic network of the pathogen, and may lead to new methods to control pathogenesis.

57

Development of Root Infection Traits in Bacterial Wilt Colonized Tomato

Tseng, T.M.P., *Iyer-Pascuzzi, A., Purdue University, Botany and Plant Pathology*

The US is the third largest producer of tomato worldwide. Tomatoes are highly susceptible to *Ralstonia solanacearum*, a soil-borne pathogen causing bacterial wilt. The pathogen invades plant roots, spreads into the crown and stem, resulting in wilting and ultimately plant death. Most work has focused on shoot system responses, while root responses to *R. solanacearum* are unclear. Our long-term goal is to identify molecular mechanisms underlying resistance to *R. solanacearum* in tomato. As a first step, we are identifying root symptoms and bacterial spatio-temporal dynamics within roots after infection in resistant (R) and susceptible (S) varieties to determine whether root infection events correlate with resistance. We are currently investigating root architecture, bacterial spatio-temporal dynamics in roots, and bacterial production of exo-polysaccharide in R and S varieties. We find that bacteria multiply faster in S roots, and that bacterial movement from roots to shoots is faster in the S variety. Further, although bacteria enter roots of both R and S tomato varieties within 5 hours post inoculation, significantly less bacteria are able to enter the R root at this early stage. Examination of root architecture showed that roots of R variety are longer, have larger area, and greater biomass 6 days after inoculation. Our data suggest that root infection traits correlate with resistance and may be used in QTL analysis to identify root resistance traits to BW.

58

Identification of Tobacco Streak Virus (TSV) in Commercial Cranberry Plantings and its Association with Berry Scarring in Wisconsin

Wells, L,* *Polashock, J., Vorsa, N., Lockhart, B., McManus, P., University of Wisconsin-Madison, Plant Pathology*

In 2012, we discovered Tobacco streak virus (TSV) in cranberry plants bearing disfigured, scarred fruit. This was the first confirmation of a virus in Wisconsin cranberries, and nothing is known about how TSV will impact yield of this woody, perennial plant. We conducted field trials in 2013 to investigate the epidemiology and impact of TSV on cranberries. TSV was detected in leaves, fruit, and pollen of cranberry plants by ELISA, reverse transcriptase PCR, and transmission electron microscopy. In 2013, we showed that TSV overwinters in cranberry plants in the field. Plants which had produced scarred, symptomatic, TSV-positive fruit in 2012 produced non-scarred, asymptomatic TSV-positive fruit in 2013. This is referred to as 'recovery' when observed in crops infected with viruses related to TSV. In 2013, we initiated experiments to determine the effects of TSV infection on cranberry yield components such as flower set, fruit set, and berry weight. In 2013, berry weight and fruit set were significantly reduced ($p<0.05$) in scarred, symptomatic, TSV-positive cranberry plants compared to non-scarred, asymptomatic TSV-negative or TSV-positive "recovered" plants. Further experiments are being conducted, but we tentatively conclude that after plants "recover," TSV does not negatively affect yield components in cranberry.

59

Oxalic Acid Production and Aggressiveness of *Sclerotinia sclerotiorum* Isolates in Soybean

Willbur, J.,* *Kabbage, M., Smith, D., University of Wisconsin-Madison, Plant Pathology*

Oxalic acid is a key pathogenicity factor of *Sclerotinia sclerotiorum*, the causal agent of Sclerotinia stem rot of soybean. Variation in isolate oxalic acid production could explain potential differences in isolate aggressiveness. Due to the broad host range of *S. sclerotiorum*, a wide range of isolate aggressiveness in the field may be a concern. A diverse collection of *S. sclerotiorum* isolates was evaluated on soybean to determine whether host specificity, based on isolate aggressiveness, exists. Oxalate production and aggressiveness assays were conducted on isolates collected from a variety of hosts in the central United States and Poland. Lesion lengths from inoculated 'Williams 82' soybeans and colony radii *in vitro* were monitored to determine isolate aggressiveness. A spectrophotometric assay was used to quantify isolate oxalate production. Surprisingly, non-soybean isolates were moderate to strongly aggressive on a susceptible soybean host. Our findings suggest that screening of *S. sclerotiorum*-resistant soybean germplasm should be performed with multiple isolates of the fungus to account for the overall diversity of *S. sclerotiorum* isolates in the field.

60

Effects of Fluopyram on Soybean Cyst Nematode and Soybean Sudden Death Syndrome

Zaworski, E.,* *Tylka, G., Mueller, D., Iowa State University*

Soybean sudden death syndrome (SDS) and soybean cyst nematode (SCN) are two of the most important diseases of soybean. Currently, resistance is the main management strategy for both diseases, and there are few other options available for each disease. Seed treatments are now an option for farmers for early

plant development. A recently registered chemical, fluopyram, has been reported as have activity against both SCN and SDS. This study tested fluopyram in different combinations with currently available seed treatment products for management of each of these pathogens separately and together. Results for the SCN alone experiment were somewhat unclear; however, plants treated with a seed treatment combination of fluopyram with clothianidin, *Bacillus firmus*, metalaxyl and trifloxystrobin had less SCN females per gram of root when compared to the same combination without fluopyram. Three runs of an experiment with SDS alone and SCN and SDS in combination were conducted. In one of these three runs, fluopyram in combination with trifloxystrobin and metalaxyl had the lowest amount of SDS foliar symptoms, but there were no significant differences among the seed treatments in any of the three runs of experiments with SDS alone and both SCN and SDS. In the SDS and SCN experiments, there were significantly fewer SCN females per gram of root in the presence with fluopyram for the contrast involving clothianidin, Bacillus firmus, metalaxyl and trifloxystrobin. These results indicate that fluopyram may be negatively affecting SCN, but we detected no such negative effects of fluopyram on SDS foliar disease symptoms in our experiments.

61

Triticum Mosaic Virus 5' Leader Acts as a Bona Fide Internal Ribosome Entry Site for Translation

Zhang, J.*, *Roberts, R., Browning, K., Tatineni, S., Rakotondrafara, A., University of Wisconsin-Madison, Plant Pathology*

Many RNA viruses rely on an internal ribosome entry site (IRES) element to maximize viral-encoded proteins, and many virues in the Potyviridae family are among them. By definition, IRES elements recruit ribosomes in a cap- and 5' end-independent manner. Few plant viruses,such as Tobacco Mosaic virus and Turnip Mosaic virus, have been reported to rely on an IRES for their translation. In contrast to most animal viruses, plant IRESes rely on very short sequences, have no defined sequence requirement, are devoid of stable secondary structure and show maximal translational activity with an open 5' end, suggesting potential organism-specific regulation of translation. Here, we investigated the potential IRES activity of the leader of the uncapped Triticum mosaic virus (TriMV, Potyviridae) RNA. The TriMV 5' leader is able to drive cap-independent translation under limiting cap-binding protein, eIF4E. We found that optimal IRES activity requires the entire 739 nt leader, which by far exceeds the typical length of plant viral leaders. When compared to previously reported plant IRESes in the context of a dicistronic construct for the translation of a downstream gene, the TriMV IRES activity was clearly 5' end-independent and retained maximal efficiency when ribosome entry from the 5' end was blocked by a highly stable secondary structure. Its activity was 100 fold higher than prototypical plant viral IRESes. Sequence and structural analysis of the TriMV 5' UTR revealed characteristical features of picornavirus IRESes, including stable structures, a polypyrimidine tract and multiple AUGs. Our preliminary results reveal that the TriMV 5'UTR appears to be the first bona fide plant viral IRES element.

62

Functional Analysis of Lateral Organ Boundaries Genes in Citrus Bacterial Canker Disease

Zhang, J.*, *Yang, H., Jones, J., Frank, W., Kansas State University*

Bacterial citrus canker, a disease complex caused by distinct, yet related strains of *Xanthomonas*, occurs in oranges, grapefruit, and limes. An isolate of *X. citri* subspecies *citri* (Xcc) strain 306 harbors four TAL effector genes, one of which, pthA4, is critical for formation of pustule lesions, which eventually erupt and undergo necrosis. Pustule formation is accompanied by enhanced bacterial proliferation in the infected tissue. Additionally, TAL effector genes pthAw from Xcc strain Aw, pthB from another causal

species, *X. fuscans* ssp. *aurantifolii* B, and pthC from *X.* f. ssp. *aurantifolii* C, are also required for pustule formation on citrus species in the respective strains. Our results indicate that distinct TAL effectors from a range of canker-causing strains of *Xanthomonas* target a single susceptibility (S) gene CsLOB1, which is a member of the Lateral Organ Boundaries (LOB) gene family of transcription factors and required for pustule formation in citrus. Here, we addressed the question whether other genes in the LOB family have the potential of serving as S genes for bacterial citrus canker. Genes related to CsLOB1 were targeted using artificial designed TAL effectors (dTALes) for promoters of CsLOB1 homologous genes and introduction of the dTALe genes into the pustule-defective strain Xcc306âˆ†pthA4. The dTALes targeting the CsLOB2 promoter restored pustule formation and promoted the increase in bacterial population. The results showed that, while only one S gene has been identified in nature, more than one LOB gene could serve as the targets of TAL effectors in citrus canker. The situation is in contrast to *X. oryzae* pathovar *oryzae*, the causal agent of bacterial blight of rice, where strains target multiple related host S genes for the SWEET sugar transporter family. The differences may reflect differences in the genetic management of the two species and the selective pressures on the pathogen populations.

63

Advancing Our Understanding of Mature Plant Resistance to Potato Virus Y in Selected Varieties Toward Improved Seed Potato Certification

Zhang, C.,* *Groves, R.L., University of Wisconsin-Madison*

Potato Virus Y, an aphid transmitted Potyvirus, continues to be an important pathogen in seed potato production due to the emergence of novel recombinant strains combined with asymptomatic infections in both plants and tubers. Mature plant resistance is a phenomenon in some varieties of potato defined by an observed reduction in disease incidence commonly associated with later season inoculations into plants. This concept can potentially be a valuable component to add to our existing PVY management programs in seed potato, especially since the majority of viruliferous aphids are active during the later portions of the growing season. Replicated field experiments were established in both 2012 and 2013 to investigate the response of four widely planted potato varieties to infection by two strains of PVY at different stages of potato development. Replicate sets of potato varieties were mechanically sap-inoculated with ordinary (PVYO) and recombinant (PVYN:O) strains during pre-flower and several post flowering stages, and compared with untreated controls. At the end of season, a portion of harvested tubers were later sub-sampled for grown-out testing in either the field or in the greenhouse. Foliar symptoms of PVY infection were evaluated after plant emergence at approximately the 5-7 true leaf stage of development. Three leaves were collected from each plant and tested via ELISA to confirm the presence of PVY originating from seed tubers. Ongoing research in the greenhouse will be completed in summer 2014 and supplemented with previous field research. Reductions in disease incidence associated with later stage inoculations were observed in all three varieties with the exception of the cultivar ‘Russet Norkotah’. Notably, the cultivars ‘Silverton’ ($F=5.5$, $df=(4,15)$, p-value=0.006) and ‘Dark Red Norland’ ($F=31.85$, $df=(4,15)$, p-value=3.6e-07) possessed the greatest overall differences between pre- and post-flower inoculations, illustrating the potential for mature plant resistance in these varieties.