2013 APS-MSA Joint Meeting

Abstracts of Special Session Presentations

Biology of Pathogens

Filling in the Gaps: How Do *Xanthomonads* Adapt to Diverse Hosts, Tissues, and Environments?

The xylan utilization system of *Xanthomonas campestris* controls epiphytic life and reveals common features with animal gut symbionts

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Phytopathology 103(Suppl. 2):S2.170

TonB-dependent receptors (TBDTs) are outer membrane proteins mainly known for the active transport of iron siderophore complexes in Gram-negative bacteria. Analysis of the genome of *Xanthomonas* species revealed an overrepresentation of these transporters. This overrepresentation is associated with the ability to exploit plant carbohydrate and we proposed the existence of specific carbohydrate utilisation systems with TBDTs (named CUT systems). Recently, we identified a CUT system involved in the utilisation of xylan, a major component of plant cell wall and the second most abundant plant polysaccharide in nature. This CUT system encompasses genes required for the degradation of xylan as well as genes for the transport and metabolism of xylo-oligosaccharides, xylose and glucuronate. Interestingly, these genes which expression is induced by xylo-oligosaccharides, are required for optimal growth on plant leaves. Part of the xylanolytic machinery of *Xanthomonas*, including TBDT genes, displays a high degree of conservation with the xylose-regulon of the oligotrophic aquatic bacterium *Caulobacter crescentus*. Moreover, it shares common features, including the presence of TBDTs, with the xylan utilisation systems of *Bacteroides ovatus* and *Prevotella bryantii*, two gut symbionts. These similarities and our results support an important role for TBDTs and xylan utilisation systems for bacterial adaptation in the phyllosphere, oligotrophic environments and animal guts.

Contribution of type III/TAL effectors to pathogenicity

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Phytopathology 103(Suppl. 2):S2.170

Most plant-pathogenic bacteria use a sophisticated protein delivery device to inject type III effectors (T3Es) into the host's cells for the benefit of the pathogen. The *French Network on Xanthomonads* studies several species of *Xanthomonas* and examines the role of T3Es during the infection process. Here, we will focus on two rice pathogens, *X. oryzae* pv. *oryzae* (*Xoo*) and *X. oryzae* pv. *oryzicola* (*Xoc*). First, the diversity within T3E repertoires of 45 strains clearly showed that both pathogens belong to closely related, but distinct, phylogenetic groups. Both pathogens comprise core and variable T3E suites that probably have distinct roles in pathogenicity and different evolutionary histories. We examined whether or not one of the differential T3Es between *Xoo* and *Xoc*, XopO, acts as a determinant of tissue specificity. Second, we monitored 41 candidate *Xoo* T3Es for their ability to translocate an AvrBs1 reporter into plant cells. Nine proteins were identified as *bona fide* T3Es. Mutations in their genes revealed that one of them, *xopR*, contributes to virulence in hybrid rice. Third, we isolated a new TAL effector that induces a rice *SWEET* gene to promote colonization of the leaf blades. Artificial TAL genes were then used to systematically evaluate the potential of *SWEET* genes to support the growth of *Xoo* in the xylem vessel. Only five phylogenetically close *SWEET* proteins which presumably act as sucrose transporters were found to provoke virulence.

Genome and transcriptome analysis to reveal adaptation to new environments and hosts

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Phytopathology 103(Suppl. 2):S2.170

Pathovars of *Xanthomonas* are interesting working models for the analysis of mechanisms of adaptation and specialization to hosts, plant tissues, niches and environments. The understanding of such mechanisms is important on practical and academic points of view since they can help explain how emergence of new diseases or of diseases in new situations may occur. Four research groups belonging to the *French Network on Xanthomonas* (FNN) have recently focused on genome and transcriptome sequencing of several pathovars of *Xanthomonas* chosen for their adaptation particularities, in a project called "Xanthomix". *Xanthomonas* strains attacking Anacardiaceae,

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

<http://dx.doi.org/10.1094/PHYTO-103-6-S2.170>
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citrus, crucifers, legumes or rice were selected to help understand adaptation processes: genetic structure revealing either convergence or divergence for causing a specific disease on a specific host ; host tissue specialization (xylem vs. mesophyll) ; virulence variation (broad vs. narrow host range); contrasted epidemic situation (endemic vs. epidemic). RNAseq experiments were performed on a large set of strains to determine the extent and the diversity of the hrp regulon. In addition, the RNAseq dataset is also being used to improve *Xanthomonas* genome annotation and gene prediction. Analysis of transcripts allowed us to scan for unknown specific and non-specific effectors as well as small non-coding RNAs and hypothesize new mechanisms that could be involved in adaptation to host and environment.

Differences in patterns of host transcriptome modulation as a measure of diversity and adaptation of TAL effector-wielding *Xanthomonas* populations

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Phytopathology 103(Suppl. 2):S2.171

Many *Xanthomonas* deploy TAL effectors to modulate host gene expression for susceptibility. TAL effectors recognize their host genomic targets via a structurally modular mechanism that allows for rapid evolution of new specificities. Degeneracy in the mechanism and the presence of duplicated, variant TAL effectors in most strains result in collateral activation of host genes inconsequential to disease. Thus, the pattern of host gene expression changes following inoculation is a potentially useful phenotype to assess pathogen strain diversity. And, changes induced in common by multiple isolates may identify key susceptibility factors. We sought to test these hypotheses by comparing by RNAseq host transcriptional responses to 11 geographically diverse isolates of *X. oryzae* pv. *oryzicola*, which causes

bacterial leaf streak of rice. The number of genes induced by a strain ranged from 1177 to 3360 and those repressed from 800 to 4300. 570 genes were induced and 370 repressed by all 11 strains. Each strain induced some genes that were not induced by any other strain. A tree based on the gene expression changes grouped the strains in agreement with their geographical grouping. Thus host gene expression changes are a valid phenotype for assessing strain diversity. Ongoing work aims to relate the gene expression changes to TAL effector content to identify adaptations important in disease.

Flagellar motility and fitness in xanthomonads

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Phytopathology 103(Suppl. 2):S2.171

Xanthomonads are plant-associated bacteria that establish neutral, commensal or pathogenic relationships with plants. These γ -proteobacteria are motile by a single polar flagellum. Motility is an important feature involved in biofilm formation, plant colonization and hence considered as a pathogenicity factor. The French Network on *Xanthomonads* studies genomics of several species of *Xanthomonas*. FNX sequenced and annotated the genomic sequence of *X. fuscans* subsp. *fuscans* strain 4834-R (*Xff* 4834-R), the agent of common bacterial blight of bean. Sequencing revealed a deletion of 34 genes (33 kbp) involved in flagellar biosynthesis, which results in abolition of motility. This feature is not solely restricted to *Xff* 4834-R, since some flagellar biosynthetic genes are missing in approximately 5% of 300 *Xanthomonas* strains tested. As a consequence, these strains are also non-motile. Interestingly, half of the *Xff* strains isolated from the same epidemic than strain 4834-R are non-motile and this ratio is conserved among strains colonizing the next bean seed generations. Isolation of such variants in natural epidemics reveals that either flagellar motility is not a key function for fitness or that some compensation occurs within the bacterial population. Fine characterization of pathogenicity and ability to aggregate in biofilms give clues to interpret maintenance of non-motile strains in populations.

Insect-Transmitted Bacterial Diseases: Passing the Gift

Recent advances in understanding the biology of the insect-transmitted bacterium, *Xylella fastidiosa*

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Phytopathology 103(Suppl. 2):S2.171

Xylella fastidiosa is a Gram-negative bacterium that colonizes the xylem tissue of its plant hosts. This pathogen causes serious diseases such as Pierce's disease (PD) of grapevine, Almond Leaf Scorch and Citrus Variegated Chlorosis among many others. *X. fastidiosa* is transmitted by xylem-feeding insects such as sharpshooters (Hemiptera, Cicadellidae) and spittlebugs (Hemiptera, Cercopidae). The majority of the work has been performed in the *X. fastidiosa*-grape-sharpshooter pathosystem, and this presentation will cover what is known about the epidemiology of PD as well as information about the possible mechanisms used by the insect during the inoculation process. Thus far, bacterial cell surface proteins and carbohydrates have been identified as important mediators of bacterial acquisition and transmission by insect vectors, and special attention will be given to summarizing what is known about the molecular mechanisms governing this interaction between *X. fastidiosa* and its insect host.

Phytophagous insects, *Salmonella enterica*, and fresh produce: A tri-trophic interaction that can make you sick

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Phytopathology 103(Suppl. 2):S2.171

Salmonella enterica is the number one cause of bacterial food-borne illness in the United States. Despite fresh produce being considered the main vehicle of food-borne illness, to date, routes of *S. enterica* contamination and mechanisms of growth in plant environments remain unclear. This presentation will focus on the potential role of phytophagous insects in the growth, survival, and transmission of *S. enterica* on and to plants. Previously, we have found that thrips can acquire and harbor elevated *S. enterica* populations from contaminated produce. Following acquisition, contaminated thrips moved the human pathogen from inoculated produce to non-inoculated plants. Movement of *S. enterica* by thrips results in the spread of the human pathogen among plants. Taken together, these results suggest that an important insect pest of agricultural crops can transmit the human pathogen *S.*

enterica. Ongoing research complements these findings with further analysis of mechanisms of insect transmission and feeding behaviors that might have a direct influence on *S. enterica* growth in the phyllosphere.

Pantoea stewartii uses distinct type III secretion systems to alternate between host kingdoms

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Phytopathology 103(Suppl. 2):S2.171

Bacterial pathogens use phylogenetically distinct type III secretion systems (T3SS) that produce needle-like injectisomes or pili for the delivery of effector proteins into plant and animal host cells. The genome of *Pantoea stewartii* subsp. *stewartii* (*Pnss*), which causes Stewart's bacterial wilt and leaf blight of maize, encodes two phylogenetically distinct T3SSs. One is an Hrc-Hrp T3SS that is essential for bacterial pathogenesis in maize. In contrast, the second T3SS (*PSI-2*) belongs to the Inv-Mxi-Spa T3SS family that is typically found in animal pathogens. Mutations in the *PSI-2* *psaN* gene, which encodes an ATPase essential for secretion of T3SS effectors by the injectisome, greatly reduced the persistence of *Pnss* in the gut of its flea beetle vector, *Chaetocnema pulicaria* (Melsh). The mutations also reduced transmission of *Pnss* to maize by beetles, but not by mechanical inoculation, indicating they did not affect pathogenesis in maize. Ectopic expression of the *psaN* gene in *Pnss* complemented the mutant phenotypes. In addition, the *PSI-2* *psaN* gene was expressed at higher levels in insects compared to maize tissues. These data indicate an important role for *PSI-2* in vector transmission of the bacteria. Thus, the Hrp and *PSI-2* T3SSs play different roles in the life cycle of *Pnss* as it alternates between its insect vector and plant host.

Erwinia tracheiphila: Getting around with a little help from my friends

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Phytopathology 103(Suppl. 2):S2.171

Cucurbit bacterial wilt, caused by *Erwinia tracheiphila* (*Et*), is a major cucurbit disease in the Midwest and Northeastern United States. *Et* is a Gram-negative, xylem-inhabiting bacterium, that affects economically important crops such as cantaloupe, cucumber, squash, and pumpkin. The bacterial wilt

cycle is closely associated with the life cycle and behavior of cucumber beetles (*Acalymma vitatum* and *Diabrotica undecimpunctata howardi*), the only known vectors of this disease. Although *Et* was among the first bacterial plant pathogens ever described, our understanding of the bacterial wilt pathosystem remains rudimentary. This presentation will contrast research performed during the early 19th century and during the past 20 years to highlight encouraging breakthroughs, key knowledge gaps, and exciting directions for future research.

Genomics of *Erwinia amylovora*–host interactions: Update and perspective

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Phytopathology 103(Suppl. 2):S2.172

Fire blight, caused by *Erwinia amylovora*, is the first plant bacterial disease discovered back in 1880s and is also the first bacterial plant pathogen for which an insect vector was demonstrated. Extensive genetic studies in the past

decade or so have demonstrated that a functional hypersensitive response and pathogenicity (*hrp*) - type III secretion system (T3SS) and its associated effectors, as well as production of the exopolysaccharide amylovanan are primary determinants in *E. amylovora* to cause fire blight disease in plant host. Insects such as honeybees have long been recognized to play a passive, but significant role in the dissemination of *E. amylovora*, especially in blossom blight phase. Many early fire blight disease management programs have also emphasized the importance of insect control. However, the exact role insect plays and the relationship between *E. amylovora* and its insect vectors are not well understood. The recent revealing of the genetic composition for more than a dozen strains of *E. amylovora* and related *Erwinia* species associated with pome fruit trees has provided new opportunities to not only re-examine the relationship with insects, but also to identify novel virulence factors during host-pathogen interactions. In this presentation, recent findings utilizing novel genomics approaches to understand *E. amylovora* virulence will be summarized and highlighted. Future perspectives for this important pathogen will also be discussed.

One Fungus, One Name: The Impact of Recent Changes in Fungal Nomenclature

Overview of changes affecting fungal nomenclature in the International Code of Nomenclature and progress of nomenclatural working groups

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Phytopathology 103(Suppl. 2):S2.172

In August 2011, the mycological world was alternatively stunned or delighted by changes to the rules governing the naming of fungi, published in early 2013 in a renamed International Code of Nomenclature for algae, fungi and plants (ICN). The most dramatic change was the formal abandonment of >100 years of dual nomenclature, and the adoption of a system where only one binomial can be validly applied to a single fungal species in all its morphological forms. The nomenclatural principle of priority is to be applied, meaning that the oldest genus and species names must be combined, irrespective of whether they originally were intended as teleomorph or anamorph names. The intricacies of this adjustment will be discussed, to provide context for the subsequent speakers, who will discuss specific examples relevant to plant pathologists. The international mycological community, led by the International Commission on the Taxonomy of Fungi (ICTF), the Nomenclature Committee for Fungi (NCF), and several other bodies, has responded by mobilizing existing taxonomically oriented Commissions and Subcommissions and establishing new Working Groups, with the goal of proposing lists of accepted names for the 10th International Mycological Congress in Bangkok, Thailand in August 2014. The activities of these groups will be summarized, with a view to maximizing participation by all interested scientists, including those whose primary focus may not be taxonomy.

Impact of ICN changes on scientific names of regulated fungal plant pathogens

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Phytopathology 103(Suppl. 2):S2.172

Scientific names communicate information about fungi. For USDA, APHIS, Plant Protection and Quarantine, names are the currency for issuing permits and the basis for pest risk assessments and quarantine decisions. Scientific names of organisms have always been subject to change based on new information and concepts. However, the change to one name for one fungal species will bring about a large number of nomenclatural changes over a very short period of time. How will the required nomenclatural changes impact plant quarantine regulations? For example, if you hold a permit under a name such as *Cochliobolus heterostrophus* and the correct name for that species is now *Bipolaris maydis*, is your permit still valid? The answer is yes because the same fungus is being permitted, only the name has changed. When two names are synonyms, the oldest name generally is used. As generic and species names compete for synonymy, committees open to all may propose exceptions based on user needs (www.fungaltaxonomy.org/subcommissions). The key to a seamless transition will be comprehensive databases of fungal nomenclature that links all names of fungi with an indication of the approved

name. Accurate scientific names can be sought at <http://nt.ars-grin.gov/fungal-databases/nomen/nomenclature.cfm>, which emphasizes plant-associated fungi, and www.indexfungorum.org/Names/Names.asp and www.mycobank.org/ for scientific names of all groups of fungi.

Merging the 2000 plus genera of Dothideomycetes

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Phytopathology 103(Suppl. 2):S2.172

The Dothideomycetes represent the fungal class with the most known plant pathogens, including more than 100 families and 2000 genera. To merge asexual and sexual genera, the type species of the respective genera have to cluster in the same, well-resolved phylogenetic clade. Once monophyly has been established, genera compete equally for priority, even though proposals have to be published to propose the use of asexual genera over that of sexual genera. In cases where the asexual genus is better known however, it can now be chosen over the sexual genus, which traditionally had priority under the previous Code. Classic examples to consider are *Bipolaris-Cochliobolus*, *Elsinoë-Sphaerulina*, *Cladosporium-Davidiella*, *Ramularia-Mycosphaerella*, *Alternaria-Lewia* and *Didymella-Phoma*, to name but a few. Priority also extends to family level, where older asexual families frequently have priority over families based on sexual genera, e.g. Cladosporiaceae versus Davidiellaceae, Phyllostictaceae versus Guignardiaceae. A special nomenclatural working group has been established under the International Commission for the Taxonomy of Fungi to publish recommendations about the integration of asexual and sexual genera in the Dothideomycetes, which should be completed later this year.

Defining the genus *Fusarium* in a scientifically robust way that best preserves longstanding use

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Phytopathology 103(Suppl. 2):S2.172

In this talk I will present the argument of a diverse group of scientists advocating a phylogenetic circumscription of the genus *Fusarium*, that includes virtually all *Fusarium* species of importance in plant pathology, mycotoxicology, medicine and basic research. This will free scientists from any obligation to use other genus names, including teleomorphs, for species nested within this clade, and preserve the application of the name *Fusarium* in the way it has been used for almost a century. Due to recent changes in the International Code of Nomenclature for algae, fungi and plants, and recent recognition of teleomorph genera that compete with this taxonomic concept, we propose that all teleomorph names associated with this group are synonyms and recommend that they not be used, specifically *Gibberella*, *Haematonectria*, *Neocosmospora*, *Geejayessia*, *Cyanonectria* and *Albonectria*. The alternative is to break the longstanding concept of *Fusarium* into nine or more genera, and remove important taxa such as those in the *F. solani* species complex from the genus, a move we believe is unnecessary. We believe these taxonomic and nomenclatural proposals will preserve established research connections and facilitate communication within and between research communities, and at the same time support strong scientific principles and good taxonomic practice.

***Pyricularia* or *Magnaporthe*? Names and genomes**

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Phytopathology 103(Suppl. 2):S2.173

The family Magnaporthaceae contains devastating fungal cereal and grass pathogens, such as *Pyricularia* (*Magnaporthe*) *oryzae* (rice blast), *Magnaporthe* (*Magnaporthe*) *poae* (summer patch), and *Gaeumannomyces graminis* (take-all). The type species of *Magnaporthe* is *Magnaporthe salvinii* (rice stem rot), which is not congeneric with *Magnaporthe* (*Pyricularia*) *oryzae* or *grisea*, based on phylogenetic analysis, morphology, and ecological characters. Therefore, a change of genus name is needed for the rice blast fungus. According to the new fungal nomenclature (Melbourne Code), the name for

the rice blast fungus should be *Pyricularia oryzae*, while the name for the stem rot is *Nakataea oryzae*, because these are the older names. Another option is to conserve the genus *Magnaporthe* for the blast fungus. A poll is set at <http://magnaporthe.blogspot.com/>. A new genus, *Magnaportheiopsis* is proposed that includes *Magnaportheiopsis* (*Magnaporthe*) *poae*, *Magnaportheiopsis* (*Magnaporthe*) *rhizophila* and *Magnaportheiopsis* (*Gaeumannomyces*) *incrusters* based on phylogenetic analysis. In addition, we performed genome sequencing for 6 species in Magnaporthaceae: *Magnaportheiopsis rhizophila*, *Magnaportheiopsis incrusters*, *Harpophora maydis*, *Nakataea oryzae* (*Magnaporthe salvinii*), *Ophioceras dolichostomum*, and *Pseudohalonectria lignicola*, in order to conduct phylogenomic and comparative genome analyses for both pathogenic and non-pathogenic members of this family.

Disease Control and Pest Management

13th I. E. Melhus Graduate Student Symposium: What's in Our Toolbox to Minimize the Risk of Plant Disease?

Soft red winter wheat yield and quality as influenced by the Fusarium head blight-Stagonospora leaf blotch complex and disease management strategies

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Phytopathology 103(Suppl. 2):S2.173

Fusarium head blight (FHB) and Stagonospora leaf blotch (SLB) may significantly reduce wheat yield and quality. In addition, FHB-affected grain may accumulate deoxynivalenol (DON), a mycotoxin that is harmful to humans and livestock. Crop loss and integrated management studies were conducted to model the combined effects of FHB and SLB on grain yield and quality and to evaluate in-field disease management and grain harvesting strategies for FHB and DON reduction. In both sets of studies, cultivars with different levels of resistance to FHB were planted and spray-inoculated to generate a range of disease levels. In the management studies, a subset of the plots of each cultivar was treated with a fungicide at anthesis and harvested with a combine adjusted to discard diseased grain. In years with moderate to high levels of FHB, both grain yield and quality decreased as FHB index increased. The rates of yield and test weight reduction (r_{yld} and r_{tw}) ranged from -29.24 to -19.59 kg/ha %⁻¹ and from -3.88 to -1.05 kg/m³ %⁻¹, respectively. Susceptible cultivars and plots inoculated with *Stagonospora* generally had the highest negative r_{yld} and r_{tw} values. Fungicide-treated plots of resistant cultivars had significantly lower mean FHB and DON and higher mean yield and test weight than untreated plots of susceptible cultivars. Plots harvested with the modified combine configuration had significantly higher mean test weight than those harvested with the default configuration.

Factors affecting the timing of abscission of peach and nectarine leaves infected with *Xanthomonas arboricola* pv. *pruni*

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Phytopathology 103(Suppl. 2):S2.173

Bacterial spot (caused by *Xanthomonas arboricola* pv. *pruni* [Xap]) is the most important bacterial disease of stone fruit in the eastern U.S. where yield limiting symptoms on fruit regularly reduce crop values in Pennsylvania. One often over-looked symptom, premature defoliation, ultimately reduces fruit quality and overall tree vigor. The objective of this study was to gain basic knowledge of the factors affecting premature defoliation as they relate to the current management strategies in PA. Data on bacterial spot severity and the time of leaf abscission were obtained on a 7 day interval in replicated field plots of four cultivars subjected to different bactericide programs in 2009. Survival analysis using the Kaplan – Meier and the accelerated failure time (AFT) models of time to leaf abscission was conducted in order to assess the effects of bactericide treatment, cultivar, leaf age, and initial disease onset on the survival of leaves infected by Xap. Of the leaves assessed for disease severity, 48% of them abscised before the end of the study. The mean time to leave abscission ranged from 41.8 to 56.3 days and was significantly ($P < 0.0001$) affected by cultivar, initial disease onset, and leaf age but not by bactericide treatment. The AFT model indicated that every additional 1% initial disease severity decreased the time to leaf abscission by 10.7%. These results indicate that strategies for bacterial spot management should focus on reducing initial disease.

Environmental and management factors associated with bacterial rots of onion in Pennsylvania

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Phytopathology 103(Suppl. 2):S2.173

Bacterial rots, including center rot (*Pantoea ananatis* and *P. agglomerans*), soft rot (*Pectobacterium carotovora* and *Pseudomonas marginalis*), and slippery skin (*Burkholderia gladioli* pv. *allicola*), are significant diseases of onion in Pennsylvania, and may result in up to 50% yield loss. Growers attempt to manage these diseases using copper fungicides and cultural practices; however, variability in marketable yields still occurs. On-farm trials were conducted in 2011 and 2012 on 28 and 26 farms, respectively, to identify potential sources of inoculum as well as production factors related to harvest disease incidence. At-planting soil, transplant, and midseason weed samples were screened for eight bacterial onion pathogens using a multiplex PCR protocol. At-planting and at-harvest soil N, leaf and bulb tissue N, soil temperature, and other factors were analyzed in a multivariate linear regression model. *P. agglomerans* and *P. marginalis* were common on and in over half of onion transplants and were most common on weed surfaces. A strong negative relationship between leaf tissue N at midseason and total harvest losses was suggested in a multivariate linear regression model, while a positive relationship was suggested between preharvest soil temperatures and disease. These results relate the importance of reducing soil temperatures, ensuring adequate soil fertility early in the season, and taking measures to reduce the impact of inoculum sources in the production system.

Integrated control of Allium white rot

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Phytopathology 103(Suppl. 2):S2.173

White rot, caused by the ascomycotina fungus *Sclerotium cepivorum*, is the most serious disease of *Allium* food crops world-wide, especially onion (*A. cepa*) and garlic (*A. sativum*). *S. cepivorum* spreads and overwinters as sclerotia, which germinate in response to *Allium* root exudates. An integrated pest management (IPM) approach is necessary for continued economic production in infested areas. Refining fungicide recommendations and combining them with sclerotia germination stimulants (SGS) (which promote sclerotia germination when no hosts are present) and biological controls are promising methods of disease control. Field studies were conducted to investigate the efficacy of combining SGS with fungicides. When fungicides were combined with an SGS, disease incidence was significantly reduced over either control method alone. By determining EC₅₀ and EC₈₀ (Effective Concentration to reduce mycelial growth by 50 and 80%) we were able to determine optimal fungicide concentration in soil, and also screen for fungicide resistance. Using HPLC-MS-ESI we determined the length of time that the most common fungicides remained in the soil root zone, and also determined that their primary mode of activity was inhibition of mycelial growth in the plant root zone. These data, combined with field studies utilizing multiple disease controls, have been instrumental in refining IPM recommendations for the control of *Allium* white rot.

An integrated approach to understanding tomato sour rot and improving disease management

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Phytopathology 103(Suppl. 2):S2.173

Geotrichum candidum is the causal agent of sour rot in tomato (*Solanum lycopersicum*) and other fresh produce. This disease is a major limiting factor of tomato production in all growing regions, including the Eastern Shore of Virginia, Florida and California. It can cause major losses in the field and during post-harvest handling, though little is still known about the disease etiology and management practices. During severe outbreaks, losses up to 25% in packaged fruit can result. Currently, there are no in-field or post-harvest fungicides labeled to reduce sour rot infections. Because of its unique classification and structure, standard fungicides are not highly effective at preventing infection establishment or transmission, leaving cultural practices

the main focus for control. This research integrates results from cultivar disease tolerance trials, molecular and *in vitro* characterization of isolates, and in-field and packinghouse epidemiological studies to better understand the tomato sour rot pathosystem so recommendations can be made to growers to reduce the impact of sour rot on commercial tomato production. Preliminary results demonstrate a significant correlation between disease incidence and specific weather conditions. Resistance to *G. candidum* was not observed during multiple growing seasons, leaving cultural controls as the most important for disease management.

Innovations in Seed Treatments for Crop Protection and Health

Development and formulation of seed treatment combinations

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Phytopathology 103(Suppl. 2):S2.174

Seed treatment products developed for today's markets are designed to cover the broadest spectrum of protection utilizing fungicides, insecticides, and nematicides. Simultaneous use of growth enhancement materials, fertilizers, herbicide safeners, and inoculant or biological control agents has also become increasingly important. The complexity of development revolves around combining many active ingredients to cover the broadest spectrum of pests. Therefore, formulation technology is driven by the need for securing acceptable physical and stability characteristics via selection of appropriate carriers to maintain shelf life over time. Assessment of formulation compatibility in mixtures with additional seed-applied products is conducted to assure acceptable use patterns. In concert with formulation development, seed safety of candidate formulations is monitored across focus crop seeds (to be labeled) over time in normal seed storage conditions. User-friendliness of the formulation in the seed plant and field planters can be pre-determined by assessing formulation dry-down on seed, conducting dust-off of formulation from the seed, and running treated seed through field planting equipment. All of these aspects must be addressed so that the major contribution that the seed treatment product provides, stand enhancement and yield benefits, is recognized by the end-user, the grower.

Adoption of new seed treatment technologies by the seed industry

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Phytopathology 103(Suppl. 2):S2.174

New seed treatment technologies bring valuable functionality such as disease & pest protection, general improved plant health & growth, and increased abiotic stress tolerance to growers. Agricultural row crop seed companies often market an integrated seed product consisting of plant genetics, GM traits, and seed treatments where the seed treatment portion is chosen to enhance and/or complement the germplasm or traits. Current seed treatment recipes may include one or more fungicides, insecticides, nematicides, growth enhancers, etc. so all components must be proven to be compatible, may not negatively impact plantability, must be safe to the seed & the environment, and must be relatively easy to apply. Seed companies must also ensure there are no freedom to operate issues because of the specific combination of treatments being applied and/or their application to specific germplasm or traits.

Enhancement of plant productivity through microbial seed treatments

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Phytopathology 103(Suppl. 2):S2.174

Microbial seed treatments have been proposed and used now for several decades for the control of plant diseases. In many cases enhanced plant growth and performance was also noted, but the mechanisms of action of action have been unknown. In fact, even the mechanisms of biocontrol have been misunderstood in many cases. Recently, there have been identified a number of microbes that are endophytic plant symbionts, including specific strains of the Ascomycete *Trichoderma*, and the Basidiomycete *Piriformospora indica*. These fungi colonize the external area of roots and at least some strains grow with the roots and provide season long benefits to crop plants. In addition, mycorrhizal fungi, and plant growth promoting bacteria in the genera *Pseudomonas* and *Bacillus*, as well as others, also colonize plant roots. These plant endophytic symbionts have qualitatively similar effects upon plants, and can induce systemic resistance to diseases;

induce resistance to plant stresses including drought, salt, and pollutants; increase plant nitrogen use efficiency (NUE), increase plant photosynthetic efficiency, and even increase antioxidants in produce. Selected strains of *Trichoderma* spp. can be applied as seed treatments and have been shown to reliably increase crop yields over hundreds of trials, and during the drought of 2012, provided remarkable benefits to stricken fields.

WITHDRAWN

Nematode-protectant seed treatments: New options for nematode management in row crops

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Phytopathology 103(Suppl. 2):S2.174

Plant-parasitic nematodes can be serious soil-borne pathogens of many field crops. In the past, these obligately parasitic worms were managed primarily by growing nonhost crops and resistant crop varieties and using soil-applied nematicides, if available. A relatively new management option for field crops is nematode-protectant seed treatments. Three different nematode-protectant seed treatments currently are available for corn, cotton and soybean farmers in the United States. The active ingredients and modes of action of products currently available include a rhizoplane bacterium (*Bacillus firmus*) that repels nematodes, a bacterial fermentation compound (abamectin) that inhibits nerve transmission in nematodes and a protein (harpin) that elicits changes in natural plant defenses. These seed treatments are designed to protect the roots from nematode feeding early in the season, not necessarily to provide season-long control. More products with different active ingredients and modes of action are likely to become available in the future. The nature of nematode protectant seed treatment products, availability and formulations, location and durability of protection and effects on nematode population densities and crop yields will be discussed.

Physiological benefits of seed treatments

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Phytopathology 103(Suppl. 2):S2.174

Seed treatments are a very important tendency adopted by agrochemicals enterprises because they are more safety for the producers, provide economic use of the products, lower agrochemical volume and better distribution homogeneity over the seed. Some agrochemicals could act on the plant cultivar changing the gene expression promoting repression or manifestation

of certain genes, inducing protein synthesis. This should promote hormonal action, and this effect could produce new proteins, more enzymatic activity and better ion transport through the cell membranes. The appearance of new proteins is the support of biochemical changes on metabolism. Increases on enzymatic activity could promote functional modifications on plant physiology increasing plant productivity. In soybean and dry bean, seed treatment with thiamethoxam increases biometric parameters, vigor, shoot and root development, enzymatic activity and some nutrients absorption. The

combination of gibberellic acid, indolebutyric acid and kinetin was efficient to increase seed germination, vigor, root development and production of soybean, dry bean, rice, peanut, sorghum and wheat. Biological tests with tomato 'Micro-Tom' were realized to better elucidate the interactions among the combinations of the bioregulators. Treatments with the combination of one, two or three bioregulators showed effect on tomato production only for the combination of three.

Diseases of Plants

Emerging Issues of Mycotoxins in Food Safety

Impacts of the Midwest 2012 drought on aflatoxin contamination of maize

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Phytopathology 103(Suppl. 2):S2.175

Aflatoxin contamination of maize occurs when grain is infected by *Aspergillus flavus*, the causal organism of Aspergillus ear rot. This disease is favored by dry, hot conditions, followed by some moisture after black layer. Aflatoxin is a potent toxin and carcinogen that is regulated by the Food and Drug Administration (FDA) who have set an action level of 20 ppb. During the 2012 growing season, much of the Corn Belt experienced severe to extreme drought conditions and concerns regarding aflatoxin contamination were high. The FDA approved temporary blending policies for aflatoxin in several states so that corn up to 500 ppb of aflatoxin could be blended for use in appropriate animal feed. The prevalence of Aspergillus ear rot was low in many states. In Iowa, mean ear rot severity was less than 2 percent in field trials. Aflatoxin levels detected in grain at harvest throughout the Midwest also were low. In surveys conducted by the Iowa and Illinois Departments of Agriculture and Land Stewardship, 6 and 20 percent of grain samples collected from local elevators in each county of Iowa and Illinois, respectively, contained >20ppb aflatoxin. The state average for Iowa was 5.5 ppb, below 21 ppb that occurred during the 1983 drought. Reports of aflatoxin-contaminated feed surfaced in early 2013 indicating incorrect sample handling may have played a role in the low levels detected at harvest.

Influences of weather on aflatoxin-producing fungi and aflatoxin concentrations in crops

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Phytopathology 103(Suppl. 2):S2.175

Aflatoxins are potent mycotoxins that reduce development, impair the immune system, and cause cancer and death. Several *Aspergillus* species produce aflatoxins during crop infection, which is greatly influenced by weather events including freezes, droughts, rainfall, and shifts in temperature. Weather also influences the average aflatoxin-producing potential of the fungi. Contamination occurs both during crop development and after crop maturation. Interactions of aflatoxin-producers with agronomic practices and weather events at specific crop stages determine the magnitude of contamination through effects on pests, crops, and timing of activities. Weather events that favor high contamination during crop development may suppress contamination after crop maturation either prior to harvest or during post-harvest handling and storage. Routine crop monitoring provides large sets of geographically referenced data useful in describing influences of weather on contamination across regions, states, and even continents. Knowledge of weather influences facilitates both better monitoring and improved cost-benefit ratios through weather-based adjustment of management strategies. Recent episodes of severe contamination on maize and cottonseed underscore the importance of weather influences and the need to adjust cropping cycles and management practices in order to avoid losses associated with aflatoxins.

Challenges in using the biopesticide AF36 in pistachio orchards to reduce aflatoxin contamination

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Phytopathology 103(Suppl. 2):S2.175

The atoxigenic *Aspergillus flavus* strain AF36 (which is used in cotton fields to reduce aflatoxin contamination of cottonseed) was evaluated in two pistachio orchards in preparation for use in commercial pistachio orchards. The AF36 strain was applied as a wheat-AF36 product (the same product and rate as used in cotton fields) in a single annual application to the soil in late June or early July. The treatment substantially increased the level of the AF36 strain in the soil in both orchards, although the wheat-AF36 product was more successful in one orchard than in the other (>95% of the *A. flavus* isolates belonged to the AF36 strain in one orchard after a single application, while the other orchard needed two applications to get >80% AF36). In addition, the level of the AF36 strain remained relatively high for at least 2 years after the last application of the wheat-AF36 product. Application of the wheat-AF36 product did not result in increased levels of kernel decay. The application of the biopesticide AF36 to 1,200 ha of commercial orchards (Experimental Use Permit) resulted in 20-45% reduction in aflatoxin-contaminated pistachio samples (average for 4 years was 40% for the 1st and 2nd harvests). Furthermore, the AF36 reduced aflatoxin-contamination by about 55% (average of 3 years) in samples from the 2nd harvest, which can be contaminated usually with high levels of aflatoxins. Various challenges of AF36 application will be discussed during the presentation.

Fumonisin production by black *Aspergillus* species in maize

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Phytopathology 103(Suppl. 2):S2.175

Fumonisin (FBs) are mycotoxins that commonly occur in maize, and typically are produced by fungal species in the genus *Fusarium*. Fungal strains belonging to *Aspergillus* Section *Nigri* also have been reported to produce FBs. Black *Aspergilli* commonly occur on maize kernels, but their role in FB contamination is not clear. We investigated the possible role of black *Aspergilli* in the FB contamination of maize kernels, by collecting 153 maize samples at harvest in Italy and analyzing the FB content and fungal infection. Isolates of *Aspergillus* Section *Nigri* were identified to species and tested for FB production in solid maize-kernel culture. We also collected and tested 137 black *Aspergillus* isolates from maize seed grown in Iowa and other parts of the U.S. Nearly all the Italian samples were contaminated by FB-producing *Fusarium* species, at levels up to 100% of kernels. In contrast, black *Aspergillus* species were found in 45/153 samples, in up to 33% of kernels. Nearly all the samples contained FBs, sometimes exceeding 200 mg/kg. Black *Aspergillus* species from Italy and the U.S. included *A. niger*, *A. tubingensis*, *A. awamori*, and *A. carbonarius*. Approximately 65% of these isolates produced FB₂ at low levels in culture. Considering the higher frequency of FB-producing *Fusarium* species and the relatively low FB production by *Aspergillus*, the contribution of the black *Aspergilli* to FB contamination seems to be sporadic, though it may be significant in some cases.

When mycotoxins come in bunches: Fumonisin production by *Aspergillus niger* in grapes

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Phytopathology 103(Suppl. 2):S2.175

Aspergillus niger is one of several black-spored *Aspergillus* species that commonly occur as epiphytes of healthy grapes, and as causative agents of black bunch rot and raisin mold. Genome sequencing of *A. niger* revealed the presence of a previously unknown gene cluster homologous to the fumonisin biosynthetic gene cluster in *Fusarium* species. This led to the discovery that *A. niger* does indeed produce fumonisins, most predominantly fumonisin B₂ (FB₂). Because of the ubiquity of *A. niger* on grapes, it is not surprising that fumonisin contamination of grape products such as wine and raisins is widespread. For example, in one study of raisins collected from five distinct vineyards, we detected fumonisin in 90% of the samples. The majority of *A. niger* strains, as well as strains of the phylogenetically distinct species *A. awamori*, produce FB₂. Analyses of the fumonisin biosynthetic gene cluster in FB₂-nonproducing strains indicate that deletion of most of the gene cluster occurs in FB₂-nonproducing *A. awamori* strains, whereas in FB₂-nonproducing *A. niger* strains, the gene cluster is intact. This distinction may be useful in monitoring FB₂-producing and -nonproducing *A. niger* and *A. awamori* populations, to predict fumonisin contamination of grapes, and to investigate seasonal variation in the distribution of these species within and among vineyards.

Aflatoxin and fumonisin contamination in corn smut (*Ustilago maydis*) galls in the field and in the grocery store

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Phytopathology 103(Suppl. 2):S2.176

Corn infected with *Ustilago maydis*, causal agent of common smut, produces galls that are used as food in certain cultures, but may be contaminated with mycotoxins. The objective of this study was to determine mycotoxin levels in common smut galls (CSG) collected at corn ear reproductive stages R1 through R6 and in commercial CGS products. A total of 108 CSG samples were collected in 2012 from corn fields and 14 CSG commercial products were purchased from grocery stores in Minnesota. All commercial products and 41 field samples were analyzed for cyclopiazonic acid, deoxynivalenol, and zearalenone. Aflatoxin was detected in 5% of CSG field samples R3 or older (up to 15 ppb) and 63% of CGS samples R2 or older contained fumonisin (up to 150.7 ppm). Sixteen field samples R3 or older contained deoxynivalenol at levels ranging from 0.1 to 1.3 ppm. Twenty field samples R3 or older contained zearalenone at levels ranging from 0.6 to 41.7 ppb. Cyclopiazonic acid was not detected in any of the field samples. Two commercial CSG products were positive for fumonisin (0.04 to 0.24 ppm) and zearalenone (6.1 to 15.1 ppb). *Aspergillus flavus* and *Fusarium verticillioides* were isolated from selected CSG field samples at 3.5×10^6 and 4.2×10^6 cfu/g, respectively. These results indicate that CSGs can be infected with mycotoxigenic fungi and contaminated with mycotoxins. The incidence of mycotoxins in commercially available products warrants further study.

Innovations in Microbial Forensics and Plant Biosecurity

New strategy, ongoing operations, and innovative projects at the National Biosurveillance Integration Center

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Phytopathology 103(Suppl. 2):S2.176

The federal government provides biosurveillance in various domains within different departments and agencies. Congress recognized the need for integrating these separate information sources by chartering the National Biosurveillance Integration Center (NBIC) within the Department of Homeland Security. NBIC works to enable early warning and shared situational awareness of acute biological events and support better decisions through rapid identification, characterization, localization, and tracking. The Center released its first Strategic Plan in November 2012, which presented a new approach to realizing the mission and vision of the NBIC, identifying core disciplines of biosurveillance integration, as well as the goals and objectives of the Center. Current operational activities of NBIC include the daily analytic processes, interaction with a variety of biosurveillance stakeholders, and execution of response support. New innovative biosurveillance projects are underway involving resources such as emergency medical systems, poison control centers, and social media analyses. NBIC is committed to collaboration and coordination across the levels of government and the private sector. With success in its mission, NBIC will support its partners' missions and provide relevant and timely information that effectively supports decision making.

Forensic epidemiology: New sensor-based plant pathogen detection: Where to look for evidence in a 300-acre crop

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Phytopathology 103(Suppl. 2):S2.176

The detection of invasive biotic plant pathogens, deliberate or otherwise, remains a key challenge in forensics epidemiology. The integration of remote sensing, Global Positioning Systems (GPS), and Geographic Information Systems (GIS) technologies, coupled with geostatistical analyses, can provide valid, science-based evidence concerning the presence and geospatial distribution of invasive plant pathogens. Using the soybean rust pathosystem as a model, we have successfully extracted pathogen-specific spatial and

temporal patterns from aerial, satellite, and ground-based sensors that can be used to detect, and accurately differentiate soybean rust, from other soybean diseases (with close to 100% accuracy). Using a GIS script that we developed called Gradient Finder, pathogen-specific spatial and temporal patterns can now be used to detect, identify, and map within-field anomalies caused by plant diseases. Using this approach, soybean rust disease foci can now be easily distinguished from disease patches caused by sudden death syndrome. Geospatial analyses can then be used to determine if spatial patterns are indicative of a natural or a deliberate introduction (i.e., a crime scene). The integration of remote sensing, GPS, and GIS technologies can also be used to deliver precise GPS coordinates as to where investigators on the ground should obtain pathogen isolates (and other evidence) for genetic analyses concerning the population structure of pathogen isolates.

Bioinformatics strategies for microbial forensics

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Phytopathology 103(Suppl. 2):S2.176

Deep sequencing (or next-generation sequencing) has altered the molecular biology landscape in many ways, including the development of the field of metagenomics. Not surprisingly, deep sequencing using metagenomics principles has been applied to diagnostics and microbial forensics. The unique DNA signatures of microbial pathogens lend themselves to forensic analysis of sequences. However, translating the effective mechanisms in place for human microbial agents (e.g. PulseNet, a database of human foodborne pathogens) to plant pathogens is not a trivial task. Relatively few plant pathogens have complete genome sequences available, and very few plant pathogens have the necessary population sequences to develop significantly useful forensic sequence-based databases. In addition, plant diagnostic clinics and networks lack the monetary and computer resources dedicated to microbial forensics in the human health arena. A diagnostic bioinformatic process, termed E-probe Diagnostic Nucleic acids Analysis (EDNA) was developed to address some of these problems, by lessening the need for input sequence and limiting the amount of memory intensive analyses. *In silico* simulations and *in planta* analyses indicated that EDNA was capable of strain typing viruses.

Invasive Threats to Palm Trees

Molecular characterization of lethal yellows and other phytoplasmas

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Phytopathology 103(Suppl. 2):S2.177

Lethal Yellow (LY) and Texas Phoenix Palm Decline (TPPD) are caused by two molecularly distinguishable phytoplasma species that have become an emerging issue across the southern U.S.; however, their distribution in this region is not well understood. These pathogens are transmitted to naïve plant hosts by phloem feeding insect vectors. The vector complexes vary throughout the region, so identification of vector and pathogen presence in different locations is needed to properly identify an appropriate regional management strategy. In this study, palms with yellowing symptoms were identified in Florida and Texas and core samples from these trees were tested for phytoplasma presence. Positive samples were molecularly characterized as LY or TPPD using multiple genes. Insects were collected from infected trees and molecularly characterized as well.

Texas Phoenix palm decline and potential vectors

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Phytopathology 103(Suppl. 2):S2.177

Texas Phoenix palm decline (TPPD) is a new disease in Florida. It is caused by a phytoplasma, related to the pathogen that causes lethal yellows (LY) of coconut. The symptoms of TPPD are similar to those of LY, but the host range and the geographic range are different. The vector(s) also might be different. LY occurs primarily in extreme south Florida, where it affects coconut palms and many species of ornamental palms. TPPD occurs in central and northern peninsular Florida, particularly in the Tampa Bay area. It affects *Phoenix* species, including *Phoenix roebelenii*, which is not susceptible to LY. It also kills *Syagrus romanzoffiana* (queen palm) and *Sabal palmetto* (cabbage palm). Neither of these species is susceptible to LY, and *S. palmetto* is the first native Florida palm known to be susceptible to a phytoplasma disease. Three planthoppers, a flatid, a cixiid, and a derbid, are potential vectors of TPPD.

Cadang-cadang disease of palm and other diseases

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Phytopathology 103(Suppl. 2):S2.177

The ornamental palm and the date palm industries are economically important in the United States. These industries are susceptible to invasive pathogens and pests. Viroid diseases of palm are one example. Coconut cadang-cadang disease, caused by a viroid, has killed over 30 million coconut palms in Southeast Asia. Tinangaja disease, also caused by a viroid, occurs in Guam. Many date palm production areas in the Eastern Hemisphere consider the red

palm weevil, *Rhynchophorus ferrugineus*, to be the most damaging pest of palm economically. The red palm weevil has been found in Aruba in the Southern Caribbean area, and more recently in the Laguna Beach area of California. The giant palm weevil, *R. palmarum*, is established in tropical America, including Central America, and has been reported in Southern California. The etiology, epidemiology, and management of these pests will be discussed.

Perspective of palm phytoplasma detection from a NPDN member lab in Texas

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Phytopathology 103(Suppl. 2):S2.177

The National Plant Diagnostic Network (NPDN) was established to enhance agricultural security through protecting health and productivity of plants in agricultural and natural ecosystems in the U.S. Diagnostic laboratories in the various states, usually associated with the land-grant university, respond and provide this service to the people of their state and beyond. Palms are grown regionally, mainly found in the southern parts of the United States. Only several NPDN labs perform palm phytoplasma detection, a PCR assay method. With the main goal of detection, universal primers are typically used to detect the presence of the pathogen. In Texas, two quarantines are imposed by the Texas Department of Agriculture: for Lethal Yellowing and Date Palm Lethal Decline (syn. Texas Phoenix Decline). In recent years, the Texas Plant Disease Diagnostic Lab has tested samples for palm phytoplasma and subjected positive detections to strain identification. To date, all samples in Texas have been identified as Date Palm Lethal Decline (a subgroup 16SrIV-D strain).

Palm diseases in Central America

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Phytopathology 103(Suppl. 2):S2.177

Cyclic epidemics of coconut and other palm diseases have been recorded in Central America. The most notable one is coconut Lethal Yellowing (LY) that has affected the region for more than a century. LY was reported in Honduras in 1996 and in a decade killed almost 95% of the highly susceptible Atlantic tall variety. Mexico and Jamaica have also experienced severe LY epidemics. In Central America the disease is presently in Honduras, Guatemala and Belize and no records exist for Panamá or Costa Rica that also have an Atlantic coast populated by susceptible varieties. This paper reports on the decade long replanting efforts in Honduras with tolerant varieties such as the Maypan hybrids in the late 1990s, Malayan dwarfs and Pacific Mexican tall and more recently, with Brazilian green dwarfs varieties. It also reports on research efforts to develop robust sampling and diagnostic methods for the LY phytoplasma and other palm diseases and to understand the possible breakdown of resistance observed in the early 2000s of the tolerant varieties. Finally, it documents other important pests and diseases such as bud rot caused by *Phytophthora palmivora* in coconut, African oil palm and other palmaceae species in the region. Specifically, it documents a recent outbreak of LY on *Thrinax raddiana* on the river banks of the Rio Platano Biosphere reserve in Honduras.

Schroth Faces of the Future: New Frontiers in Mycology

Evolutionary informatics to wage peace with fungi for a sustainable future

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Phytopathology 103(Suppl. 2):S2.177

Evolutionary informatics continues to grow in prominence in mycology. This is due both to the necessity of molecular identification of fungi and to the tractability of fungal genomes to data collection and analysis. Modeling of molecular sequencing evolution has enabled a fungal tree of life and the inference of key genomic innovations in fungal ecologies. In my research in this area, I have investigated the interplay between horizontal gene transfer, genome organization and natural selection that has shaped the metabolic diversity among fungi. I will present vignettes and patterns from this work that point to exciting new opportunities for understanding the nature of fungal

disease emergence. I will speculate on the implications of further advances in biological information detection for the future of mycology from both a basic and applied perspective. To illustrate these implications, I will detail hypothetical analytic pipelines linking molecule-based field observations to applied solutions in a near future when evolutionary informatics begins to realize its potential. Examples will include maintaining the health of commodity plants, optimization of biofuel production, and bioremediation polluted ecosystems. In the end, I will propose how this technological evolution can foster the transition to a paradigm of predictive and preventive fungal management in human-centric ecologies.

Migration and evolution of *Phytophthora* plant pathogens in the age of globalization

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Phytopathology 103(Suppl. 2):S2.177

Phytophthora plant pathogens have been the subject of research for more than 150 years, yet we still have much to learn. The series of events that led to the emergence of *P. infestans* as a global pathogen in the 1840's are debated and

new virulent genotypes continue to move between growing regions. The sudden oak death pathogen, *P. ramorum*, changed the way that we think about forest *Phytophthora*, and the many new *Phytophthora* species that are being discovered in forest environments continue to challenge conventional wisdom. While regulations, improved crop breeding, and disease management may control the damage from *Phytophthora* pathogens in the short-term, managing these pathogens in the long-term will require knowledge of their global movement, how and why some *Phytophthora* emerge as damaging pathogens, and the biology of endemic *Phytophthora* in their native ecosystems. My research examines these questions using several *Phytophthora* species. Recent work on the multiple introductions and migration of *P. ramorum*, ongoing research on the emergence and re-emergence of *P. infestans*, and new research on forest *Phytophthora* will be presented. These examples will be used to illustrate my vision of how population genetic and evolutionary approaches can be used to understand past events and prepare for future challenges.

Measuring oomycete biodiversity in aquatic, forest, and agricultural ecosystems: Culture-based and metagenomic approaches

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Phytopathology 103(Suppl. 2):S2.178

The fungal-like oomycetes include a number of devastating pathogens, such as the notorious potato late blight agent, *Phytophthora infestans*; it is not surprising then that most research has focused on host-pathogen interactions, with relatively little study concerning the natural diversity of saprotrophic species. This knowledge gap has important ramifications on our estimates of oomycete biodiversity, as well as on our understanding of the evolutionary history of this important group. The goal of this study is to estimate oomycete biodiversity from several habitats, including aquatic environments, undisturbed forest soils, and highly managed agricultural settings. While our culture-based surveys have relied on various baiting techniques, it is known that these methods lead to biased estimates of biodiversity as they favor fast-growing organisms and those producing zoospores. We have therefore developed a metagenomic approach to more thoroughly sample diversity from

the different environments. We are currently verifying the mitochondrial COI locus as our sequencing target; previous studies have shown that this locus is able to discriminate among closely related species, and provide phylogenetic signal at several taxonomic levels. We expect that this combination of culture-based and sequence-based methods will enhance our understanding of oomycete ecology and evolution, and perhaps give us more insight into the roles of certain species in the outbreak of disease.

Evolutionary history and genetic diversity of *Didymella bryoniae* and *Phoma caricae-papayae*, pathogens of cucurbits and papaya

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Phytopathology 103(Suppl. 2):S2.178

The patterns of genetic diversity within species, as well as the evolutionary processes contributing to the formation of new species, are exciting areas of investigation in mycology. Using a multilocus sequencing approach we have discovered that *Didymella bryoniae*, a pathogen of cucurbits, is composed of three genetic lineages. Unexpectedly, one of the lineages is genetically and morphologically indistinguishable from a pathogen of papaya, *Phoma caricae-papayae*. Current research is aimed at determining host specialization among lineages and inferring the ancestral host and divergence time of the lineages using coalescent analyses. Additional studies are focused on investigating sources of inoculum and understanding the underlying population structure of *D. bryoniae* on watermelon in the southeastern U.S. We developed 20 microsatellite markers to assess diversity within and among fields and to track the genetic similarity of populations from potential inoculum sources, including seed, wild cucurbit reservoirs, seedling transplants, and plant debris in the field. Isolates were collected from transplant seedlings, early field epidemics, and four field populations and genotyped. Similarity among isolates and diversity within and among field populations will be discussed. This work highlights the use of molecular systematics and population genetics to elucidate genetic identity among previously unassociated fungi and to solve practical disease problems in the field.

Status and Challenges in Identification and Diagnosis of Graminicolous Downy Mildews

Biology of downy mildews from gramineaceous crops

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Phytopathology 103(Suppl. 2):S2.178

Oomycetes include numerous pathogens that cause significant damage to a large number of agricultural and forest commodities. Among these are the graminicolous downy mildews that infect a number of important agricultural crops, most notably maize, millet, sugarcane and sorghum, in tropical and subtropical regions. In the tropics, the eleven most recognized Oomycete species capable of infecting these important hosts belong to the genera *Peronosclerospora*, *Sclerospora*, and *Sclerophthora*. Ten of the species are pathogenic on maize and capable of causing significant disease losses. A comparative summation of the lifecycles, geographic distribution, host ranges and efficacy of current control measures will be presented.

Downy mildews from gramineaceous crops in North America and those of regulatory concern

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Phytopathology 103(Suppl. 2):S2.178

In terms of economic impact, *Peronosclerospora sorghi* may well be the downy mildew pathogen most threatening to U.S. corn and sorghum production. While optimal reproduction of *P. sorghi* occurs on susceptible sorghums, the pathogen also infects maize and on rare occasions the disease has spread to corn grown as far north as Indiana. Discovery in south Texas a decade ago of a strain of *P. sorghi* resistant to metalaxyl seed treatment and subsequently of a new pathotype that reproduces on a formerly resistant commercial sorghum hybrid, raises the possibility that the disease could again lead to significant crop losses. Today, the downy mildew most often observed in maize is 'crazy top', caused by *Sclerophthora macrospora*. Although extra height makes infected plants quite noticeable and the ears are replaced by a mass of proliferating shoots, typically only rare plants in waterlogged areas of a field show symptoms, making disease losses relatively inconsequential. Concern that exotic downy mildews introduced either accidentally or deliberately would severely damage corn production in the U.S. led to two

species, *P. philippinensis* and *Sclerophthora rayssiae* being placed on the list of plant pathogenic select agents. While sources of resistant maize are available in Asia, the current status of resistance to either of these oomycetous pathogens in popular U.S. cultivars is unknown. Alternate hosts, including sugarcane and sorghum make *P. philippinensis* of greatest concern.

Environmental metagenomics of oomycetes and downy mildews

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Phytopathology 103(Suppl. 2):S2.178

Spore traps have been used by plant pathologists for many decades but their use has been hampered by difficulties in identifying the spores captured by the samplers. PCR based technologies have provided capability of detecting and quantifying certain species on the traps but the range of species that can be detected remains limited. Next generation sequencing is providing the capability of generating thousands and even millions of sequences per samples. By sequencing PCR amplicons generated from somewhat universal primers, it is possible to get a very wide range of fungal sequences, including oomycetes, from a sample. The number of sequences obtained from one species gives some indication of quantity. The analysis of such data clearly shows that there are many gaps in GenBank in order to match most sequences to species level, and this is particularly obvious with obligate pathogens such as downy mildews.

Taxonomy and phylogeny of graminicolous downy mildews

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Phytopathology 103(Suppl. 2):S2.178

Graminicolous Downy Mildews (GDM) are neglected oomycete diseases that can have devastating effects on subsistence farmers in the semi-arid tropics and subtropics. But they are also posing a threat to global agriculture as many of the species have been reported to be pathogenic to maize and some have been reported as pathogens of sorghum. The taxonomy of GDM is still in flux, owing to original descriptions that often only include either the sexual or the asexual stage of the pathogens. Phylogenetic investigations have revealed that indeed several otherwise highly specific GDM are able to infest maize, which include lineages of unclear taxonomic affinity and new species. For efficient quarantine regulation, these lineages need to be described and discriminated from other species. As a consequence, two new species of *Peronosclerospora*

have recently been described from Australia, *P. sargae* and *P. australiensis*, the latter of these is also pathogenic to maize. Other species still need to be defined taxonomically. In addition, phylogenetic investigations have shown that most of the distinct phylogenetic lineages have a regional distribution and could probably be contained in their native range, if appropriate measures were taken.

Status, challenges and tools for identification and diagnosis of *Peronosclerospora* and *Sclerophthora* of regulatory concern for graminicolous crops

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Phytopathology 103(Suppl. 2):S2.179

Graminicolous Downy Mildew (GDM) diseases caused by the genera *Peronosclerospora* (13 spp.) and *Sclerophthora* (6 spp. and 1 variety) are poorly studied but destructive diseases of major crops such as corn, sorghum, sugarcane and other graminoids. Eight of the 13 described *Peronosclerospora* spp. are able to infect corn. In particular, *P. philippinensis* (= *P. sacchari*), *P. maydis*, *P. heteropogonis*, and *S. rayssiae* var. *zeae* cause major losses in corn yields in tropical Asia. In 2012 a new species, *P. australiensis*, was described based on isolates previously identified as *P. maydis* in Australia; this species is now a pathogen of major concern. Despite the strong impact of GDM diseases, there are presently no reliable molecular methods available for their detection. GDM pathogens are among the most difficult Oomycetes to identify using molecular tools, as their taxonomy is very challenging, and little genetic sequence data are available for development of molecular tools to detect GDM pathogens to species level. For example, from over 15 genes used in identification, diagnostics or phylogeny of *Phytophthora*, only ITS1 and *cox2* show promise for use with GDM pathogens. Multiplex/multigene conventional and qPCR assays are currently under evaluation for the detection of economically important GDM spp. Scientists from the USA, Germany, Canada, Australia, and the Philippines are collaborating on the development and testing of diagnostic tools for these pathogens of concern.

Ecology and Epidemiology

Filling the Gap: Understanding Factors Driving Expanding Distributions of Plant Viruses

Virus-vector-host plant interactions: Factors that influence the spread of hemipteran-borne plant viruses

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Phytopathology 103(Suppl. 2):S2.179

Several biotic and abiotic factors may alter the spread of plant viruses in the field. Among them, changes in temperature and concentration of CO₂ may affect the life cycle and dispersal of insect vectors of plant viruses, that will affect the temporal and spatial distribution of virus diseases. Furthermore, the presence of natural enemies may alter the behavior and dispersion patterns of insect vectors of plant viruses. We will show how the presence of aphid natural enemies may alter the distribution and spread of aphid-borne viruses. Furthermore, viral pathogens can influence the behavior and fitness of their insect vectors, by two different ways: directly-mediated by the presence of the virus in the vector's body- and indirectly-mediated by changes occurring in the plant as a consequence of infection. It has been proposed that plant viruses may modify the behavior of their insect vectors adapting to each type of virus-vector relationship in a way that transmission efficiency is optimized. We will show examples on how plant viruses can alter the behavior and performance of their vectors to enhance their own spread.

The panacea of host resistance genes: The inadvertent selection of resistance-breaking begomoviruses

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Phytopathology 103(Suppl. 2):S2.179

Begomoviruses (*Geminiviridae*) are ssDNA viruses are widely distributed in ruderal plant species, and cause diseases of cultivated species in which they have emerged as pathogens with the recent, rapid expansion of monoculture crop production. The upsurge and/or exotic introduction of agroecosystem-adapted *Bemisia tabaci* haplotype vectors are thought to have influenced recent begomovirus diversification. Plant virus diversity has largely been studied at the species or family levels, on large geographical scales, or based on virus-satellite richness within diverse host plants. Diversity studies of begomoviruses addressing within viral species genetic structure, and its relation to geographic distance or potential gene flow barriers, and/or to the health of the larger community in relation to biomass production, remain largely unstudied. Here we evaluated begomovirus-satellite sequence diversity in a cotton-vegetable agroecosystem in which emergent viral-satellite complexes have overcome resistance in cotton within a few years following the development of 'resistant cultivars'. Viral genome-beta satellite datasets were analyzed with respect to phylogeography and demographic changes over time. Genetic diversity and gene flow analysis employs F statistics and Bayesian clustering to estimate diversity.

Evolutionary genetics factors underlying the emergence and spread of plant RNA viruses

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Phytopathology 103(Suppl. 2):S2.179

An emerging virus is the causative agent of an infectious disease whose incidence is increasing following its first appearance in a new host population as a result of long-term epidemiological changes. The sources of emerging viruses are reservoir species in which the pathogen is already well established. Over recent years, agriculture has been seriously compromised by a succession of epidemics caused by new viruses that switched host species (e.g. *Tomato torrado virus*), or new variants of classic viruses that acquired virulence factors (e.g. necrogenic *Cucumber mosaic virus*). Although viral emergence has been associated with ecological change or agronomical practices bringing in contact reservoirs and crop species, the picture is much more complex and results from an evolutionary process in which the main players are the changes in ecological factors, virus' genetic plasticity, host factors required for virus replication (including active defence mechanisms), and a strong stochastic component. I will review relevant evolutionary genetics concepts useful to understand emergence (e.g. mutation and recombination rates, *GxE* interactions and epistasis on mutational effects, metapopulation dynamics). I will also discuss whether emergence requires adaptation to the new host at early stages of infection or it is a stochastic process involving transmission of a pre-existing strains. Finally, I will discuss why certain types of viruses are more prone to emerge than others.

Specialty crop clean plant centers—Managing plant health through pathogen screening and distribution of plant materials

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Phytopathology 103(Suppl. 2):S2.179

Clean plant centers for specialty crops exist around the world with the mission of screening plants for economically significant pathogens, performing therapy for those pathogens as needed, and maintaining collections of each crop which are available to commercial nurseries for propagation and eventual sale to growers. This strategy is invaluable in improving the health of crops like grapes, citrus, berries, hops, sweet potatoes, roses and many others. In recent decades as Experiment Station budgets have declined, funding for these centers became increasingly scarce. Fortunately, the 2008 Farm Bill dramatically changed this situation by funding a National Clean Plant Network. The NCPN has served not only to fund clean plant centers throughout the United States but also to create an expert network of the scientists who manage these programs. Similar techniques are used throughout the network to avoid introduction of pests and diseases, to detect pathogens in new accessions, to perform therapy to eliminate pathogens, and to maintain and distribute propagating material.

Free trade, fair trade, safe trade: The role of plant pathology in filling regulatory gaps

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Phytopathology 103(Suppl. 2):S2.180

The globalization of agricultural markets and the increasing volume and speed of trade increase the potential for the introduction and spread of harmful organisms. The cryptic nature of many pathogens and the challenges associated with detecting them in either trade or the environment, combined

with large information gaps associated with the biology, behavior, and identification of pathogens create significant challenges for regulators trying to formulate appropriate policies for safe trade. The World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures and the International Plant Protection Convention form a harmonized international framework which disciplines regulatory decisions with an evidence-based approach. This emphasis on science creates both opportunities and threats which plant pathologists need to understand and navigate in order to fill regulatory gaps and avoid having unintended effects on trade, protection, politics, or science.

Functional, Evolutionary, and Ecological Diversity of Wood Decay Systems

Mechanisms of wood decay inferred from recent genome investigations

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Phytopathology 103(Suppl. 2):S2.180

Common inhabitants of woody debris and forest litter, 'white-rot' fungi can efficiently mineralize all plant cell wall components including the recalcitrant lignin. The unique physiology these fungi has attracted recent interest, as plant feedstocks are increasingly viewed as a potential source for high value, small molecular weight products. Extracellular oxidative and hydrolytic enzymes of white-rot fungi are thought to be involved in lignin and cellulose degradation, respectively. The model white-rot fungus, *Phanerochaete chrysosporium*, simultaneously degrades lignin and cellulose, whereas the phylogenetically related polypore, *Ceriporiopsis subvermispore*, selectively depolymerizes lignin with relatively little cellulose degradation. In contrast, 'brown-rot' decay fungi such as *Postia placenta* rapidly depolymerize cellulose but the lignin remains as a modified polymeric residue. Small molecular weight, diffusible oxidants, such as hydroxyl radical, have been implicated, and the non-enzymatic Fenton reaction ($\text{H}_2\text{O}_2 + \text{Fe}^{2+} + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{Fe}^{3+} + \cdot\text{OH}$), has been repeatedly linked to brown-rot decay. Nevertheless, mechanistic aspects of brown-rot remain obscure. Comparative genome studies of wood decay fungi are being published at an increasing rate. Together with transcriptome and proteome data, these investigations are defining the key genes and processes in lignocellulose degradation. Recent advances will be discussed and areas of uncertainty highlighted.

Co-expression analysis of *Phanerochaete carnosae* genes during growth on heartwood from deciduous and coniferous wood

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Phytopathology 103(Suppl. 2):S2.180

Coniferous trees are the predominant form of land plant biomass in the Northern hemisphere and are among the most recalcitrant biomass resource to bioprocess technologies. The white rot fungus *Phanerochaete carnosae* has been isolated almost exclusively from conifers, while most characterized white-rot species, including *Phanerochaete chrysosporium*, were mainly isolated from hardwoods. A contributing factor to growth on extractive-rich conifers could be the comparatively high number of P450 monooxygenases encoded by *P. carnosae* (266). However, like many sequencing efforts, the genome sequence of *P. carnosae* comprises many genes that encode proteins with unknown function, which might also contribute to conifer decay. Accordingly, the aim of the current study was to analyze co-expression patterns of genes that encode predicted lignocellulose-active enzymes and those that encode proteins with unknown function. Specifically, *P. carnosae* was cultivated on ball-milled trembling aspen and white spruce and total mRNA was isolated at five growth stages for Illumina sequencing. Co-expression patterns revealed distinct clustering of lignin-active and carbohydrate-active enzymes, and several of these clusters also contained genes predicted to encode transcription factors and proteins with unknown function. By mining co-expression profiles of *P. carnosae* genes, our aim is to identify new proteins and regulatory networks relevant to biomass conversion.

Wood decay in the Ozark Highlands: Variation across species, space, and time

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Phytopathology 103(Suppl. 2):S2.180

One of the largest terrestrial biological stores of carbon is wood. As plants senesce and decay, this carbon returns to the atmosphere. Studies have shown environmental conditions affect decay rates; however, we know much less about how anatomical and chemical construction of plant species affects these rates, especially how different constructions control colonization by different fungi. We established wood decay plots in ridgetops and valley bottoms in the Ozark Highlands with logs from 21 species of woody plants. These plants vary in their carbon fractions and nutrient contents, as well as size and type of water conducting vasculature. After 1 year of decay, species lost on average 27% of their biomass and by 3 years of decay species lost on average 52% of their biomass. But, variation in mass loss across species was considerable (16-92%). This variation was better predicted by plant species membership than location in the landscape. The underlying plant traits best explaining these differences were chemical rather than anatomical. Fungal communities varied considerably among plant species leading to differences in fungal enzyme expression. Hydrolases were higher in logs with high mass loss, while differences in peroxidases were less clear. Taken together, construction of plants while they are living places strong controls on who colonizes them and how they decay when they are dead.

Wood-rotting fungi have a dark history: Evidence from the fossil record

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Phytopathology 103(Suppl. 2):S2.180

Structurally preserved wood is relatively common in the fossil record. Traditionally, only pristine specimens are kept for xylotomical studies, while other more poorly preserved material is discarded. Certain ill-preserved fossil woods, however, represent valuable sources of information on fungal activities. In this contribution, we review the record of wood-rotting fungi from the Paleozoic and Mesozoic. Evidence comes from several groups of plants, including Devonian progymnosperms, Carboniferous seed ferns, and Permian to Jurassic conifers. Thin sections have turned out to be superior over other techniques (e.g., acetate peels) in assessing the extent and three-dimensionality of the fungal infection. Moreover, they are best suited to reveal structural and morphological details of both the hosts and fungi. Recent studies of wood decay in the fossil record also utilize multiple types of microscopic techniques to determine diagnostic features of the interactions, including subtle evidence such as hyphal erosion troughs. These studies underscore the importance of morphological features in accurately comprehending wood decay processes in the fossil record, and thus provide valuable information that can be used in concert with molecular data to reconstruct the evolution of wood-rotting fungi and their diverse interactions with plants.

Wood decay in extreme environments

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Phytopathology 103(Suppl. 2):S2.180

Decomposition of wood takes place in all environments facilitating carbon and nutrient cycling. In temperate and tropical regions, wood decaying Basidiomycota that cause a white or brown rot type of wood degradation are the dominant fungi. Decay may be rapid and many different forms of attack can be found. In environments where extreme conditions impose restrictions on biodegradation, such as arid sites or cold Polar Regions, different decay fungi are found causing a soft rot type of degradation. This symposium presentation will discuss investigations of wood decay in different environments with micromorphological observations used to elucidate the type of degradation and culturing, followed by sequencing the ITS region of

rDNA, to identify fungi associated with the decay. Studies in Antarctica and the Arctic reveal the occurrence of soft rot as the main cause of wood decay in Polar Regions. At many of these sites, the wood has been introduced by early explorers of the North and South Polar Regions and it has historic significance that warrants preservation. This decay is often associated with a diverse group of *Cadophora* species that appear to have widespread circumpolar distribution. Although fungi in the Ascomycota causing soft rot normally dominate these extreme environments, some Basidiomycota, such as *Hypochniciellum*, *Jaapia*, and *Sistotrema*, were encountered but their distribution was limited.

Novel industrial lignocellulose-degrading enzymes

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Phytopathology 103(Suppl. 2):S2.181

The last decade has been characterized by unprecedented efforts in developing techno-economically viable industrial enzymes optimally designed to depolymerize plant polysaccharides into fermentable sugars for production of renewable liquid fuels and chemicals. These efforts have resulted in new tools, technologies, and products which played, and continue to play, a critical role in further advancing this rapidly emerging industry. Over the past 3 years, as a member of the Novozymes BioEnergy Management Team and the Head of Protein Chemistry in the US, I have been responsible for managing an excellent team of scientists tasked with the selection of best enzyme and fungal strain candidates for the development of the leading Novozymes biomass-degrading enzymes products which are now commercialized under the names Cellic® CTEC3 & Cellic® HTEC3. This has been a unique and rewarding experience which leveraged on the tremendous potential of Novozymes enzyme biotechnology for the advancement of cellulosic biofuels. This presentation will cover the latest in technology development of novel lignocellulose-degrading enzymes and will walk the audience thru a short

history of the science and technology of cellulases and hemicellulases, a history I have been part of for the past 20 years of my career as a scientist, a manager, and an entrepreneur.

Diversification of wood decay systems in early evolution of Agaricomycotina

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The Agaricomycotina have evolved the most powerful enzymatic apparatus to attack wood. Copy number evolution in gene families encoding enzymes involved in lignocellulose decomposition shows a significant correlation with the evolution of nutritional strategies, with considerable diversification in white rot lineages and gene loss in brown rot lineages. Recent studies suggest that white rot, i.e. the ability to break down both lignin and cellulose, evolved around the same time as the divergence of Agaricomycetes and Dacrymycetes (certain jelly fungi), but detailed genome-based analyses of the early events of the evolution of nutritional strategies have been impaired by the lack of dense sampling of genomes around these nodes of the fungal tree. In current work, we are extending the sampling of genomes, focusing on the earliest-diverging orders. We are presently analyzing whole genome sequences for *Exidia glandulosa* (Auriculariales), *Sistotremastrum suecicum*, *S. niveo-cremum* (Trechisporales), *Calocera viscosa*, *C. cornea* (Dacrymycetales), *Daedalea quercina* (Polyporales) and *Sistotrema brinkmannii* (Cantharellales). We will incorporate these species into existing phylogenomic datasets of single-copy genes to infer a species tree, which we will use to reconstruct evolution of the wood-decay apparatus. The goals of our analyses are to refine our understanding of the evolution of the wood-decay system during a period of significant ecological innovation in Agaricomycotina.

Fungal Ecology Beyond Boundaries: From Communities to the Globe

An experimental test of the functioning of arbuscular mycorrhizal symbioses across scale

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Phytopathology 103(Suppl. 2):S2.181

A major goal in mycorrhizal ecology is to better understand the factors that determine plant growth response to mycorrhizal fungi. Most studies have focused at the individual plant level using +/- mycorrhizal treatments under greenhouse settings, with response outcomes ranging from parasitism to mutualism. Often the direction and magnitude of these outcomes are unpredictable. Here I will present data from two experiments conducted at different scales (one at the local level, and the other at the biome level), which are designed to better understand how environmental variables influence plant growth response. Locally, by conducting plant growth response experiments along environmental gradients (soil nutrients, pH, and moisture) it is clear that plants respond to mycorrhizal fungi differently. Under optimal conditions, the outcome of the symbiosis is parasitism. However, under more stressful conditions, the outcome is mutualism. This indicates that the mycorrhizal symbiosis may best be regarded (from a phytocentric viewpoint) as an insurance policy. At the biome level, we find similar trends. Plant growth response to mycorrhizal fungi differs across ecosystem types, with stronger positive responses in more extreme conditions, especially in arid and semi-arid ecosystems. Collectively, these results give us a more predictable understanding of mycorrhizal functioning in a variable environment.

Fungal ecology in a community context: Nectar microfungi interacting with bacteria, plants, and birds

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Phytopathology 103(Suppl. 2):S2.181

Individual ecologists tend to study a narrow group of organisms, and fungal ecologists are no exception, yet most organisms live in a complex web of interactions with many other species, even those that are remotely related. The need to look beyond taxonomic boundaries is well recognized, but rarely met. I will present an example showing the importance of interactions among remotely related organisms. It involves fungi that inhabit the floral nectar of a bird-pollinated shrub in California. Fungi disperse among flowers by hitchhiking on birds and other animals, and use nectar sugars and amino acids

for growth. Fungi are not the only nectar inhabitants, however. Bacteria are also common in nectar, and evidence indicates that fungi and bacteria negatively affect each other in nectar. The outcome of these interactions depends on the order of species arrival, with early-arriving species often excluding late-arriving ones via resource pre-emption and habitat modification. Evidence further indicates that nectar microbes can reduce seed production and nectar consumption by animals, thereby weakening plant-animal mutualism. This effect is species-specific, caused only by bacteria, and not fungi, likely due to differential modification of nectar chemistry. These findings suggest that the fungi are intimately connected with the bacteria, the plants, and the animals, and that the ecology of any of these remotely related organisms cannot be understood in isolation of any other.

Does nitrogen availability affect ectomycorrhizal fungal communities at the regional scale?

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Phytopathology 103(Suppl. 2):S2.181

Nitrogen availability has previously been shown to play an important role in structuring ectomycorrhizal (ECM) fungal communities at small spatial scales, such as within single forest plots or across local gradients. In order to test whether nitrogen availability also influences fungal communities across broad spatial scales, we examined the relationship between nitrogen and ectomycorrhizas in part of a European biomonitoring network of pine forest plots. Results of this analysis suggest that nitrogen availability has a measurable impact on ECM fungal community composition and diversity across the forest plots, despite the presence of additional complex environmental gradients such as geographic location and climate. This dataset was also used to investigate what other factors may be important in structuring ECM fungal communities in pine plots, and explore the potential use of indicator species to predict nitrogen-induced shifts in fungal communities.

Modeling fungal decomposition pathways across scales

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Phytopathology 103(Suppl. 2):S2.181

Linking community composition to ecosystem function is a central goal of ecology. We tested the hypothesis that functional type of soil fungi is a better predictor of decomposition capability than evolutionary history (i.e.

phylogenetic relatedness). To test this hypothesis, we studied measured activity of 10 different extracellular enzymes produced by 55 model species of fungi on sterilized leaf litter in laboratory microcosms. To determine if individual taxa drive decomposition pathways in nature, we plan to compare microcosm data with field data on extracellular enzyme activity and fungal community composition in *Pinus* dominated soils collected from across North America. Certain decomposition capabilities differed among phylogenetic groups of fungi. Cellobiose-, hemicellulose-, and protein-targeting enzymes all differed significantly among orders of fungi ($P = 0.0003$, $P = 0.0499$, $P = 0.018$, respectively). By contrast, activity of all extracellular enzymes was significantly higher in litter colonized by saprotrophs compared to ectomycorrhizal species. However, within closely related pairs of fungi, species identity was a better predictor of decay capability than functional type ($P < 0.0001$). Collectively, our results indicate that while functional type is a better predictor of decomposition niche than phylogenetic relatedness of fungi, species identity is the major determinant of decomposition capability.

Aboveground-belowground linkages: Extrapolating local to global fungal biodiversity

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Phytopathology 103(Suppl. 2):S2.182

Despite their importance to natural and agroecosystems, the magnitude and structure of fungal diversity on Earth are highly uncertain. We carried out an exhaustive molecular census of fungi in soil in a relatively simple ecosystem – black spruce forests of Interior Alaska. We achieved the first saturated census of soil fungi in a forested system and show that the fungus:plant ratio is at least 17:1 and is regionally stable. A global extrapolation of this ratio would suggest 6 million species of fungi, as opposed to leading estimates of 616,000 to 1.5 million. We have carried out similar surveys along transects from the low to the high arctic, and find that fungus:plant ratios are not consistent at this wider geographic scale, making global extrapolation problematic. In both our boreal and arctic studies, we find strong niche-partitioning among closely related fungi. In this presentation, we will touch on the biogeographic, functional and evolutionary implications of these findings.

Strangers in a new land: Do *Alnus* and *Salix* trees associate with different ectomycorrhizal fungi outside their native ranges?

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Phytopathology 103(Suppl. 2):S2.182

The ectomycorrhizal (ECM) fungal communities associated with *Alnus* and *Salix* species has been well characterized in their native ranges, but no belowground work has been done on the ECM fungi associated with either host genus in New Zealand. Despite sharing similar life histories aboveground, the ECM fungal communities associated with *Alnus* and *Salix* appear to differ dramatically. *Salix* hosts a diverse range of ECM fungal species dominated by lineages commonly found on other host genera. In contrast, *Alnus*-associated ECM fungal communities are notably species-poor and dominated by host specific species. To determine the extent to which these patterns hold in areas where both genera are non-native and co-invading riparian habitats, we sampled the ECM root tip communities associated with *Alnus* and *Salix* species from both the North and South island of New Zealand. We found that the ECM fungal community associated with *Salix* was more

diverse than associated with *Alnus*, and despite consistent intermingling of roots, there was very little overlap in ECM species composition among host genera. In addition, the ECM fungi associated with both *Alnus* and *Salix* in New Zealand appear to be strongly dominated by non-native species. This study, along with previous work on ECM host invasions, suggest that ECM fungal community patterns found in New Zealand are largely congruent with those found in the native ranges of diverse host species.

The relative influence of evolutionary history, climate, and space on current distributions of arbuscular mycorrhizal fungi at the global scale

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Phytopathology 103(Suppl. 2):S2.182

The biogeography of arbuscular mycorrhizal (AM) fungi will depend on both the host-specificity of AM fungi and the biotic, environmental, and spatial controls over AM fungal distributions. We determined how plant host phylogeny, plant community type, soil type, and climate influenced the global distributions of AM fungi by analyzing 18S DNA sequences in GenBank with host descriptions. We hypothesized that if AM fungi are constrained to specific plant hosts, their distributions should mimic host distributions. Otherwise, we expected that AM fungal distributions would largely reflect environmental factors. We further predicted that dispersal limitation would consistently affect AM fungal distributions as these taxa have large, animal-dispersed spores. Overall, 85% of AM fungal taxa only associated with one plant host. Of AM fungi that associated with multiple hosts, 17% had phylogenetically clustered host associations. Across sites, plant community composition explained 18% of the variation in AM fungal community composition. By comparison, environmental filtering and dispersal limitation each explained 16% and 12%, respectively. More AM fungal-plant association data is needed, as undersampling can bias results towards host specificity. Nevertheless, if AM fungi are more specific to host than expected based on historical paradigms, this warrants further consideration in our examination of both current and future AM fungal distributions.

From the rhizosphere to the biosphere: A continental-scale look at fungal diversity in North American pine forests

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Phytopathology 103(Suppl. 2):S2.182

While ecologists have long recognized the importance of scale on ecological processes, fungal communities have primarily been studied at small-scales, focusing on deterministic processes. Understanding how macroecological processes shape fungal communities is in part hindered by the lack of datasets spanning large spatial-scales and the absence of distributional data for most fungi. To rectify this knowledge gap we use next generation sequencing of the internal transcribed spacer region of the nrRNA genes to survey soil fungi across North American pine forests, spanning a diverse range of climates from Florida to Alaska. We also conducted extracellular enzyme assays to test for relationships between form and function of the fungal community across the North American continent. Fungal communities were highly variable, but showed strong evidence of regional geographic structure and geographic region explaining significant proportions of community variation. In contrast, measures of potential soil enzyme activity appear determined by within sample resource availability. These results suggest that macroecological processes are a major determinant of fungal community structure and that there is high functional redundancy in soil fungal communities across North America. This work demonstrates that increasing the scale of observation is critical to a complete understanding of the ecological dynamics of soil fungi.

Responses of Plant-Symbiotic Fungi to Climate Change: Diversity, Distribution, and Function

Fungal community responses to discrete precipitation pulses under altered rainfall intervals

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Phytopathology 103(Suppl. 2):S2.182

Climate models for central United States predict greater variability in precipitation with likely impacts ecosystem properties. We analyzed soil fungal biomass and communities in the long-term Rainfall Manipulation Plots (RaMPs) experiment before, 1 day, and 5 days after scheduled rainfall in June and September, 2011. RaMPs experiment includes ambient – precipitation distributed following a rain event – and increased interval treatments – accumulated water distributed at 50% greater intervals. As the RaMPs permit scheduled, discrete precipitation events, it served perfectly to focus on long- and short-term dynamics in soil. Our data show that the communities responded to pulses and that these responses were more pronounced in the extended interval treatment: fungal biomass increased within 5 days post-pulse, but only early growing season when adequate moisture was available. The biomass responses corroborate community richness and diversity, which decline in response to the pulse. The latter seem counterintuitive, but

indicate detection of a greater number of copies of dominant, responsive OTUs. Biomass, richness, and diversity were dynamic, whereas the composition overall was stable through the pulse. Our data highlight the rapid community dynamics and the context dependency of fungal responses to pulse events.

Climatic drivers of fungal endophyte distributions and their impacts on plant drought resistance

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Phytopathology 103(Suppl. 2):S2.183

Fungal endophytes living within plant leaf tissues can directly affect plant physiology and growth, which may be particularly important under stressful conditions such as drought. Yet little is known about the effects of endophytes on plant function or what forces structure their communities. To address these questions, we isolated, identified, and tested the function of endophytic fungi in *Panicum* grasses across a steep precipitation gradient. Rainfall was the most important predictor of endophyte community composition across the gradient in two dry years, with mean annual precipitation and current spring rainfall together explaining between 38.8% and 50.1% of the observed variation in fungal distributions. There did not appear to be dispersal limitation in these horizontally transmitted fungi based on a lack of spatial effects. When endophytes isolated from drier and wetter regions of the gradient were paired with grass seedlings under dry and well-watered conditions in the greenhouse, fungal origin did not consistently reflect their effects on plant water loss. Fungi from drier sites reduced plant water loss on average ($P = 0.012$), but there was substantially greater variation among individual taxa. While species sorting largely explains local endophyte community composition, their function in symbiosis is not predictable solely from local environmental conditions.

Climate change, endophyte symbiosis, and ecosystem engineering in dune ecosystems: Can fungi affect how plants build dunes?

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Phytopathology 103(Suppl. 2):S2.183

Dune ecosystems along the Atlantic Coast and in the Great Lakes are built by the pioneer grass species, *Ammophila breviligulata*, which can host an epichloid fungal endophyte. While endophyte symbiosis is uncommon in the Great Lakes region, the majority of plant material available for dune restorations has the endophyte. We investigated the effects of fungal symbiosis on dune ecosystem engineering by *Ammophila* in the context of climate change. We imposed both reductions and additions of growing season precipitation on plots of *Ammophila* at Leelanau State Park, Leelanau, MI. Within a plot, *Ammophila* plants were either artificially inoculated with the endophyte or sham-inoculated, in a factorial design. Symbiosis with the endophyte improved plant growth, even more so than water addition. However, the presence of the symbiotic fungus did not improve plant resilience to drought, as evidenced by the lack of a significant interaction between the endophyte treatment and precipitation manipulation. Endophyte

symbiosis additionally caused faster sand accumulation, suggesting fungal benefits to dune engineering.

Mycorrhizal feedbacks with global change: An ecophysiological perspective

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Phytopathology 103(Suppl. 2):S2.183

It has been documented numerous times that ectomycorrhizal (ECM) fungal community composition shifts in response to changes in the environment, including temperature, moisture, nutrient availability, and atmospheric CO₂ level, to name a few. Despite the influence of ECM fungi to the carbon economy of their hosts and to a variety of ecosystem functions in forests, little is known about the variation in the ecophysiological response of these fungi to different global change factors. This limits our ability to understand how ECM fungal community shifts in response to global climate change might impact important ecosystem functions. We can, however, build on some studies from the past decade that exposed species-level responses of ECM fungi to environmental factors to predict how these fungi might affect ecosystem functions as our global climate changes now and into the future. I will share implications for ecosystem functions with two examples, including a suite of studies suggesting that elevated CO₂ levels shift the ECM fungal community toward more rhizomorphic species and research I conducted that revealed that ECM fungi vary in their respiration responses to changes in temperature at short (physiological) and long (evolutionary) time scales. Additionally, I will point out where we need more research to strengthen our predictions on mycorrhizal feedbacks with global change.

Fires as global change: Responses by mycorrhizal fungi

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Phytopathology 103(Suppl. 2):S2.183

Fires are a pervasive disturbance in forest ecosystems, and warmer and drier climates are predicted to increase wildfire occurrence. Fires consume aboveground vegetation and alter soil conditions. Thus, they are likely to strongly affect mycorrhizal fungi. We used a fire chronosequence in upland boreal forests of interior Alaska (1, 7, 12, 24, 55, ~90, and ~100 years post-fire) to examine mycorrhizal responses to fires. We measured root colonization by arbuscular mycorrhizal fungi and ectomycorrhizal fungi along the chronosequence. In addition, we used pyrosequencing of 18S fungal DNA to examine changes in fungal community composition following wildfires. Root colonization by arbuscular mycorrhizal fungi did not differ across the fire chronosequence sites. In contrast, ectomycorrhizal root colonization was significantly reduced at recently burned sites and required at least 15 years to return to pre-fire levels. These root colonization data were corroborated by our pyrosequencing data. DNA sequences from ectomycorrhizal taxa were common in organic soils from mature boreal forests, but were scarce in recently burned soils. Taken together, our results suggest that boreal forest fires negatively affect ectomycorrhizal fungi, and the recovery of ectomycorrhizal communities following fires can require at least a decade. The long-term effects of boreal wildfires on ectomycorrhizal fungi may impede forest recovery and alter the assembly of post-fire plant communities.

Molecular/Cellular/Plant-Microbe Interactions

Exploring Genomic and Molecular Mechanisms of Host-Parasite Interactions for Crop Protection

The Peanut Genome Consortium and Peanut Genome Sequence: Creating a better future through global food security

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Phytopathology 103(Suppl. 2):S2.183

The competitiveness of peanuts has been threatened by losses in productivity and quality. The U.S. Peanut Genome Initiative (PGI) was launched in 2004, and expanded to global in 2006 to address these issues beginning with marker development and genetic map improvement. Ultimately, the peanut genome sequencing project was launched in 2012 by Peanut Genome Consortium, a coalition of international scientists and stakeholders that guide and implement research in Peanut Genome Project (PGP) as an integral program of International Peanut Genome Initiative (IPGI). IPGI has over 135 members in 20 countries at 79 institutions and is a committed step by the world-wide peanut research community to meet the needs of the peanut industry. PGP goals are: 1) a high quality tetraploid reference genome sequence, 2) high throughput genome and transcriptome characterization of tetraploid and diploid, 3) phenotypic trait association with genetic haplotypes, 4) interactive bioinformatic resources. The outcome will enable molecular breeding for enhancing peanut genetic improvement. The large size (2.8 Gb) and allotetraploid nature of peanut genome are challenges for peanut assembly. Therefore an integrated approach has been deployed that complements whole genome sequencing with BAC x BAC, GWAS with Recombinant Inbred Lines, and emerging sequencing technologies to bring

the assembly together. SNP discovery also will contribute to a high-density genetic map for chromosome level assembly.

Population structure and genomics of the stripe rust pathogen and interactions with its host plants

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Phytopathology 103(Suppl. 2):S2.184

Puccinia striiformis f. sp. *tritici* (*Pst*) is an obligate biotrophic fungus causing stripe rust, an important wheat disease worldwide. The whole genome and several cDNA libraries of *Pst* were sequenced. Molecular markers developed based on cDNA and genomic sequences were used to study *Pst* population structures. Studies using SSR markers and specific gene sequences revealed heterokaryotic variation, mutation, and somatic hybridization as mechanisms for *Pst* evolution. Global and U.S. population studies identified two distinct genetic clusters and revealed gene flow among countries and continents. Population studies with both *Pst* samples from wheat crops and *Puccinia* aecial samples from barberry plants in the U.S. Pacific Northwest did not support any role of alternate hosts to stripe rust epidemic and *Pst* variation. However, we have developed a system with *Berberis* spp. to study genetics of *Pst* virulence. The system has a potential to improve *Pst* genome assembly. We used the transcriptomics approach to identify *Pst* genes involved in the infection process in both compatible and incompatible interactions. Similarly, we also used the microarray technique to determine defense genes involved in different types of resistance controlled by various resistance genes. With the genotyping by sequencing technique, we are studying both host and pathogen genes involved in the plant-pathogen interactions simultaneously.

Global efforts in managing rice blast disease

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Phytopathology 103(Suppl. 2):S2.184

Rice blast disease caused by the fungus *Magnaporthe oryzae* is the single most destructive disease threatening global food security. Resistance (*R*) genes to *M. oryzae* are effective in preventing infections by strains of *M. oryzae* carry the corresponding avirulence (*AVR*) genes. Effectiveness of genetic resistance is often limited by humidity and temperature during the rice growing season. Consequently, blast disease is managed with an array of tools including the use of *R* genes, fungicides and sophisticated cultural practices. Since the first identification of *R* genes decades ago, over 100 blast *R* genes have been mapped in clusters on rice chromosomes. Among them, 20 major and 2 minor blast *R* genes have been molecularly characterized, and the molecular basis of resistance and molecular markers for some critical *R* genes has been developed for rice breeding. However, *AVR* genes in *M. oryzae* have been known to be highly unstable and changes due to transposition, point mutations, and deletions of *AVR* genes have been observed in field and laboratory isolates worldwide. Consequently, resistance based on one or few *R* genes can be quickly broken down. Additionally, fungicide resistance, and alteration of humidity, air and water temperature due to global warming present additional serious challenges for rice blast management. Ideas for global collaboration on battling blast will be presented.

Mycotoxins produced by the rice false smut pathogen

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Phytopathology 103(Suppl. 2):S2.184

Ustiloxins and ustilaginoidins are two kinds of mycotoxins produced by the rice false smut pathogen *Villosiclava virens* (anamorph *Ustilagoidea virens*). Ustilaginoidins belong to bis-naphtho-gamma-pyrone derivatives. Ustiloxins are cyclopeptides containing a 13-membered cyclic core structure. Ustiloxins are regarded as the tubulin-binding compounds involved in the dynamics of the microtubule network. In this study, ustiloxins A and B as

two main mycotoxins were determined conveniently by LC-ESI-MS in the water extract from rice false smut balls which were mostly composed of the chlamydospores and mycelia of the pathogen. Both ustiloxins A and B in the water extract were also quantitatively analyzed by HPLC. The optimum condition for ustiloxins A and B extraction was methanol concentration as 10%, extraction pH value as 6, material-to-solvent ratio as 1:30 (g/mL) and extraction times as 3 by using an L_{18} orthogonal array design with three levels and four factors. Under the optimum extraction condition, the content of ustiloxins A and B in rice false smut balls was 0.80 mg/g and 0.57 mg/g, respectively, on a dry weight basis. The adsorption/desorption characteristics of ustiloxins A and B on twenty macroporous resins were evaluated. Two resins namely SP207 and SP700 were tested to have good adsorption/desorption properties for ustiloxins A and B. Dynamic adsorption/desorption tests were further carried out to optimize the process parameters.

Mechanisms and management of carbendazim resistance in *Gibberella zeae*

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Phytopathology 103(Suppl. 2):S2.184

In China, *Gibberella zeae* is dominant pathogen causing wheat head blight. Carbendazim (MBC) has been widely used to control this disease since the 1970s. However, the resistance to MBC was popular in *G. zeae* currently. Though the MBC-resistance was controlled by one major gene and involved in the mitotic division, no mutation in the target β -tubulin was found, different from other filamentous fungi. To identify the MBC-resistance mechanism of *G. zeae*, other members of tubulin genes were analyzed. Alteration at amino-acid codon 17 or 167 or 198 or 200 in β_2 -tubulin was found to corresponding to the different phenotypes of MBC-sensitivities. Deletion/complementation of the β_2 -tubulin gene as well as mononucleotide displacement and affinity of MBC binding tubulins validated the point mutation conferring resistance of *G. zeae* to MBC. It is interested to find that MBC-resistance mutation leads to the increase in expression of deoxynivalenol (DON) biosynthesis genes. Compared to wild-type MBC-sensitive strain, resistance strain produced twice of DON in infected grains. Novel chemical 2-cyano-3-amino-3-phenylacrylic acetate is recommended as a *Fusarium* specific fungicide to control MBC-resistance *Fusarium* head blight.

Primary research progress on the resistance of rice varieties against two rice viruses transmitted by small brown planthoppers (SBPH) in China

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Phytopathology 103(Suppl. 2):S2.184

Rice stripe virus (RSV) and *Rice black-streaked dwarf virus* (RBSDV) transmitted by small brown planthopper (SBPH) are the most serious virus diseases of rice in China. Rice stripe disease, caused by RSV, first occurred in the 1990s and then broke out in Eastern China recently. Although most japonica cultivars were susceptible, a japonica rice cultivar, Zhendao 88, is resistant to rice stripe disease. We demonstrated that Zhendao 88 was resistant to virus but was weakly tolerant to the vector, SBPH using non-preference and antibiosis test. Preliminary inheritance analysis suggests that the resistance in Zhendao 88 was conditioned by a single dominant gene on the rice chromosome 11 within 4.7 cM of a simple sequence repeat (SSR) marker RM229 and a rapid amplified polymorphic DNA (RAPD) marker OPO11. Rice black-streaked dwarf disease, caused by RBSDV has caused serious damages in Eastern China since 2007. We have recently developed a simple and robust method to identify resistant rice germplasm. Consequently, a RBSDV resistant variety Tetep was identified using this new method. Preliminary inheritance analysis suggests that resistance observed in Tetep was conditioned by multigenes. Progress on the identification of user friendly genetic markers for marker assisted selection to manage both virus diseases will be presented.

Interaction Between Plants and Human Pathogens

A microbe is a microbe: What plant pathologists can and do to contribute to food safety research and outreach

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Phytopathology 103(Suppl. 2):S2.184

Plant pathologists are key players in efforts to address food safety concerns. While such concerns include a wide range of issues, outbreaks of human pathogens on fresh produce have become particularly problematic in the past decade. With expertise in plant-microbe interactions, microbial ecology, and plant disease epidemiology and control, plant pathologists are uniquely suited to make substantial gains in our understanding of interactions between plants, human pathogens and other microorganisms. Decades of research on postharvest diseases are highly applicable to the analysis and prevention of

outbreaks of *E. coli* and *Salmonella* on fresh produce. Plant pathologists are learning how human pathogenic bacteria survive, multiply and interact with other microorganisms in plant environments. Whether or not tactics for pre-harvest management of plant pathogens affect the introduction and persistence of human pathogens is being addressed. Are the points of entry and dispersal of human and plant pathogens in crops related, and is co-management a reasonable goal? Plant pathologists are in an excellent position to convey knowledge to crop producers, their traditional clientele. Producers surveyed recently overwhelmingly preferred to receive food safety information in-person from university extension. Plant pathologists, with their colleagues in other disciplines, are contributing meaningfully to the ultimate goal of ensuring a safe and healthful food supply.

Human enteric bacteria transmission to leafy greens by flies

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Phytopathology 103(Suppl. 2):S2.185

Synanthropic flies are known mechanical vectors of human pathogens, primarily in hospital and restaurant settings. However, the role of flies in transmission of pathogenic bacteria to food crops is largely unknown. Using blow flies, *Phormia regina*, and house flies, *Musca domestica*, vector competence and the transmission parameters of two GFP-tagged bacteria, *Escherichia coli* O157:H7 and *Salmonella enterica* was tested. Adult flies were given access to cow manure inoculated with 10^6 cfus/gr *E. coli*, *Salmonella*, or buffer control, then transferred to lettuce plants. Plants were homogenized and 100 μ l aliquots plated on selective media. Analysis of recovered GFP-expressing colonies revealed that house flies and blow flies transferred more *E. coli* O157:H7 to lettuce than *S. enterica*. Blow flies were also tested for acquisition of each pathogen per unit time. Flies were given precise 10 sec and 30 sec acquisitions on manure contaminated with bacteria or control buffer, then homogenized and plated as above. Blow flies acquired significantly more *E. coli* O137:H7 than *S. enterica*, regardless of acquisition time. When pathogen-exposed blow flies were given two 30 second inoculation periods on lettuce discs, flies deposited approximately the same number of *E. coli* O157:H7 cells as *S. enterica* cells. These data suggest greater *E. coli* adhesion to fly tarsi and mouthparts compared to *Salmonella*, but equal release rates to the lettuce surface for the two pathogens.

Hanging on and hanging out, *Salmonella*'s life in roots and leaves

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Phytopathology 103(Suppl. 2):S2.185

Salmonellosis is the most common bacterial food-borne illness contracted by Americans, and contaminated produce is the most likely source of infection. *Salmonella enterica* is an animal pathogen that colonizes plants, meaning *S. enterica* can adhere, replicate, and persist on plants presumably to return to an animal host. The human illness which results from colonization of plants increases the urgency to understand the mechanisms used by enteric pathogens in plant niches. *Salmonella* colonization of plants can be considered a model system to better understand adaptation strategies an animal pathogen can use to survive in multiple niches, and thus maximize the probability of encountering a susceptible host. Recent research suggests that phyto

pathogens increase the persistence of enteric human pathogens in plants. Continued effort is necessary to discern the benefit provided by phyto

Plant immunity against human pathogens

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Phytopathology 103(Suppl. 2):S2.185

Enterohemorrhagic *Escherichia coli* and *Salmonella enterica* appear to be the most common causal agents of food poisoning associated with the consumption of fresh leafy vegetables. These human pathogens are not proven to be plant pathogens yet. Nonetheless, under certain conditions these bacteria can survive on and penetrate into plant tissues causing serious food borne disease outbreaks. In this study, we sought to identify components of the plant immunity system that are regulated in the presence of either *E. coli* O157:H7 or *S. enterica* serovar Typhimurium SL1344. During the epiphytic phase, O157:H7 induces strong and lasting stomatal closure, whereas SL1344 induces a transient closure in both the model plant *Arabidopsis* and lettuce. These findings raise the possibility that not only plant pathogens (some pathovars of *Pseudomonas syringae* and *Xanthomonas campestris*), but also some human pathogens (SL1344) have evolved mechanisms to subvert stomatal defense to enter plant tissues and survive endophytically. It is equally possible however, that SL1344 is able to evade recognition by the plant immune system. Using high throughput technologies, we have also observed that SL1344 does not induce very strong apoplastic immunity as compared to O157:H7. Recent results highlighting the differential plant responses induced by these two human pathogens will be discussed.

A food safety perspective on the interactions of enteric viruses with plants

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Phytopathology 103(Suppl. 2):S2.185

Produce safety has reached a higher level by integrating the knowledge of food microbiology with that of plant pathologists in an attempt to address key issues concerning pathogen interactions with plants. While a great deal of recent research has focused on bacterial interactions, specifically with *E. coli* and *Salmonella*, human viruses are also linked to disease associated with plant commodities. Human noroviruses cause 58% of gastroenteritis with the U.S. and are suspected to be the leading cause of foodborne illness. Additionally, noroviruses account for over 50% of all foodborne illnesses transmitted by leafy greens and raw agricultural commodities. Research questions on survival, attachment, persistence and transfer of viruses to plants have been addressed with the use of norovirus surrogates like murine norovirus and Tulane virus. In general results indicate that enteric viruses persist on leafy greens, attach to leaf surfaces, hide within stomatal openings, and may be transmitted to humans on healthy plant surfaces. Efforts to better understand interactions of enteric viruses with plants have led to research questions concerning the plant immune response and how leafy greens might respond to enteric virus contamination.

Interactions and Mechanisms of Symptomless Plant Symbioses

Parallels between mutualism and pathogenesis: A comparison of lichen and pathogenic symbioses

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Phytopathology 103(Suppl. 2):S2.185

Symbiotic interactions involving fungi are ubiquitous and form a continuum from mutualism to parasitism. The two ends of this spectrum were thought to generate disparate evolutionary patterns, but current understanding of these symbioses suggests that both interactions coincide in many mechanisms (such as defense and infection) and genomic signatures. Additionally, there is an ongoing debate on evolutionary signatures of these interactions (i.e., "arms race" versus "mutualistic environment"). The lichen symbiosis has been mostly absent from this debate because of the lack of knowledge about the genes at play in establishing and maintaining this type of symbiosis. Here we use nine lichen mycobiont genomes encompassing Lecanoromycetes, Lichino-

mycetes, Eurotiomycetes and Arthoniomycetes, to explore the genomic signatures of symbiosis and compare these patterns with more than 30 Pezizomycotina genomes encompassing pathogenic (on animals and plants), non-pathogenic, and beneficial interactions. Specifically, we compare patterns of repetitive sequences and transposable elements as potential mechanisms to promote adaptation, as well as gene density and evolutionary rates as putative hallmarks of symbiosis. Complementarily, we use congruence of mechanisms between beneficial and parasitic interactions as a proxy to predict genes of importance in mycobiont-photobiont interactions using key genes in host-pathogen interactions.

Dual mutualist-antagonist dynamics of grass endophytes

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Phytopathology 103(Suppl. 2):S2.185

Systemic, seed-borne endophytic fungi, such as *Neotyphodium* species, inhabiting cool season grasses have been traditionally viewed as plant mutualists. The main mechanism for mutualistic effects is the fungal production of alkaloids that increase resistance to herbivores, pathogens and seed predators, and generally increase host competitive abilities. Yet accumulating evidence shows that the effects of endophytes can range from

mutualistic to antagonistic, especially in native grass populations. This disparity in the effects of seed borne endophyte on their hosts has several explanations. First, most studies of endophyte effects on host are short term. Recent studies show that the direction and strength of endophyte interactions with perennial hosts changes ontogenetically and lifetime fitness effects are challenging to measure. Second, recent molecular genetic evidence shows that even asexual endophytes like *Neotyphodium* are remarkably variable across and within populations. For example, variation in alkaloid genes among endophytes leads to highly variable interaction outcomes depending on the presence and type of herbivores and abiotic conditions such as soil nutrients. Third, endophytic alkaloids often cascade from herbivores upward to higher trophic levels. Natural enemies may be more affected by the alkaloids than are their herbivore prey, and thus plant defense via endophytic alkaloids may be thwarted. I provide examples of these three scenarios.

Obligately lichen-associated fungi in the lichen microbiome: How did they get there and what are they doing?

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Phytopathology 103(Suppl. 2):S2.186

Approximately 20% of described fungi are lichen-forming, including nearly half of the Ascomycota. Recent studies indicate that lichens harbor complex microbiomes composed of an astounding diversity of prokaryotic and eukaryotic microorganisms, some of which appear to be obligately lichen-associated. Lichenicolous fungi are obligate fungal parasites of lichens that have been collected and described since the 1700s. Over 1800 species have been described throughout the Ascomycota and Basidiomycota, but their overall species diversity remains uncharted territory. They are broadly distributed phylogenetically in fungal clades with and without present-day lichens, representing seven classes and twenty orders in the Ascomycota and four classes and eight orders in the Basidiomycota. Species include both phycoparasites and mycoparasites that vary in virulence; they may or may not exhibit obvious symptoms. In some cases, they appear to represent transitional forms intermediate between mutualistic and parasitic/other modes of nutrition. Recent studies have begun to elucidate the direction, tempo and mode of these transitions and the evolutionary processes that drive them.

Cell-cell signaling coordinates endophytic lifestyle of *Xylella fastidiosa*

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Phytopathology 103(Suppl. 2):S2.186

The bacterium *Xylella fastidiosa*, while capable of causing diseases to important plants such as grape and citrus also commonly occurs as an endophyte in symptomless plant species. Even in host plants most cells occur

in small numbers in the xylem vessels that it colonizes and it exhibits strong cell density-dependent behaviors that tend to self-limit its population size in plants, presumably to avoid the cell death that is associated with the plugging of vessels due to bacterial over-growth. *X. fastidiosa* employs a quorum sensing system that utilizes fatty acid signal molecules that increase in local concentration in proportion to cell numbers in a vessel to suppress the production of type IV pili enabling twitching motility and extracellular enzymes that enable transit between xylem vessels and also contribute to cell multiplication by liberating consumable carbon sources, while enhancing expression fimbrial and afimbrial adhesins that restrict movement within the plant, but which are required for acquisition by, and hence transmission to, new host by insect vectors. *X. fastidiosa* might thus be considered a common endophyte whose growth and movement in certain host plants is not efficiently repressed at high cell densities.

Hemibiotrophy: The *Magnaporthe oryzae*-rice interaction

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Phytopathology 103(Suppl. 2):S2.186

Hemibiotrophy in the rice blast system involves an extended biotrophic stage in which *Magnaporthe oryzae* colonizes living rice cells. Individual invaded cells are no longer viable when the fungus moves to the next cell. New understanding has emerged from live-cell imaging of the fungus expressing fluorescently-labeled effector proteins during biotrophic invasion. The fungus secretes cytoplasmic effectors, which accumulate in biotrophic interfacial complexes (BICs) and are translocated into the rice cytoplasm. BICs localize in front of the tips of filamentous hyphae that enter rice cells, and remain beside the first bulbous pseudohyphal invasive hyphal cells as these hyphae continue growing. In contrast, secreted apoplastic effectors remain in the extracellular space between the fungal cell wall and the rice plasma membrane, where they uniformly outline the entire invasive hypha. Chemical treatment that disrupts the conventional ER-Golgi secretion pathway and analysis of pathogen mutants that fail to express exocyst components or a t-SNARE suggest that *M. oryzae* possesses distinct secretory mechanisms for cytoplasmic and apoplastic effectors. Analyses of candidate effectors that localize where hyphae cross the plant cell wall, together with correlative light and electron microscopy, are underway to test the hypothesis that the fungus targets rice pit fields containing plasmodesmata for effector trafficking and cell-to-cell movement.

New Horizons in the Cell Biology of Fungi

The glowing guts of *Neurospora crassa* hyphae

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Phytopathology 103(Suppl. 2):S2.186

Most models for fungal growth have proposed a directional traffic of secretory vesicles to the hyphal apex, where they temporarily aggregate at the Spitzenkörper (SPK) before they fuse with the plasma membrane (PM). In *Neurospora crassa* we have found that vesicles carrying the biosynthetic nanomachines for the fibrillar components of the cell wall concentrate at the SPK in an orderly manner. In contrast, the PM H⁺-translocating ATPase (PMA-1) accumulates at the PM at distal regions (>120 µm) and in completely developed septa, but not at the tip, indicative of a distinct secretory route independent of the SPK occurring behind the apex. PMA-1 is delivered via the classical secretory pathway (ER to Golgi) to the cell surface, where it pumps H⁺ out of the cell, generating a large electrochemical gradient that supplies energy to H⁺-coupled nutrient uptake systems. Earlier predictions suggested that the H⁺-ATPase in *N. crassa* is deficient or inactive at the apex but abundant distally. Our results show not only that H⁺-ATPase is deficient at the apex, but also that exocytosis of PMA-1-GFP containing vesicles does not contribute to PM growth, supporting the existence of an alternative exocytic mechanism, which does not involve full vesicle fusion.

Live cell imaging of the dynamic actin cytoskeleton during growth and development in *Aspergillus nidulans*

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Phytopathology 103(Suppl. 2):S2.186

Polarized growth of filamentous fungi requires actin. Until recently there was no reporter for the live cell imaging of actin dynamics that revealed actin cables as well as patches in filamentous fungi. The Lifeact reporter used here couples an actin binding domain with GFP and RFP for visualization of the cytoskeleton. The sub-apical collar of endocytic actin patches and contractile rings at septation sites were both revealed by Lifeact and their dynamics agreed with previous results. Additionally, actin rings were found to mark the nascent site of lateral branch formation. Actin cables spanned the long axis of the hyphae near the tip and were found to be associated with the Spitzenkörper. A complex array of actin cables was associated with growing tips and exhibited two distinct dynamic patterns. In slow growing, still developing germ tubes and lateral branches an apically localized array of cables was found in the cell apex. Cables formed in the apex and exhibit treadmill toward the sub-apical region where they ultimately dissociated. In faster growing, mature hyphae the actin complex (sub-apical actin web – SAW) was localized 10-20 microns behind the apex. Cables were stable on the distal face of the SAW but were dynamic off of the proximal face of the SAW with cables appearing to grow and retract to and from the cell apex.

Revealing fungal communication modules by genomics, population genomics, and genome wide association studies in *Neurospora crassa*

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Phytopathology 103(Suppl. 2):S2.186

Cell communication is essential for coordination of development and reproduction in all organisms. In filamentous fungi, such as *Neurospora crassa*, asexual spore germlings are capable of undergoing directed growth and cell fusion, which contributes to fitness via rapid colony establishment. Germling communication in *N. crassa* is dependent upon the rapid oscillation of a MAP kinase protein (MAK2) and the SO protein to germling tips showing chemotropic interactions. To identify new components of germling communication, we exploited both genomic and population genomic resources available for *N. crassa*. Utilizing RNA-seq and phosphoproteomic data, we identified potential targets of MAK2 kinase, one of which oscillates to germling tips with MAK2 during germling communication. In an alternative approach, we performed genome wide association (GWA) on ~100 *N. crassa* isolates from a single population to identify genes that affected the complex, quantitative trait of conidial communication and fusion. We identified a number of genes whose inheritance was associated with the trait; one strongly so. Deletion of seven of these genes strongly affected germling communication and cell fusion. We believe that ours is the first GWA study of a wild microbe; GWA of wild populations is an attractive and less labor-intensive method to identify genes associated with quantitative traits and is particularly well suited to fungi.

Following the compartmentalization of filamentous fungus

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Phytopathology 103(Suppl. 2):S2.187

Septum formation and cytokinesis in eukaryotes share three basic steps: selection of the division plane, assembly of an actin contractile ring (CAR) and the constriction of the CAR coupled with the invagination of plasma membrane at these sites. To maintain an orderly sequence of these steps, spatial cues and temporal controls must play a crucial role. Although the key players of septal development have been identified, the precise spatiotemporal distribution of these components still needs further research. To study temporal distribution, we performed live-cell imaging of *N. crassa* strains carrying GFP labeled proteins known to be involved in different stages of

septum formation. For site selection, we used the landmark protein Bud4-GFP. To monitor actin ring formation, we followed the starting Rho4 module (Rho4-GFP, Bud3-GFP and Rgf3-GFP), responsible for the activation of formin (Bni-GFP), which in turn promotes actin polymerization. We imaged the actin cytoskeleton by means of five actin binding proteins (ABPs): tropomyosin, coronin, fimbrin, Arp3 and Lifeact. We measured the times at which the proteins appeared in relation to the internalization of plasma membrane labeled with FM4-64. In this way we were able to construct a timeline of the proteins involved in septum formation. For the most part the sequence was the same as in other organisms, but surprisingly, we found that actin cables are formed at future septation sites much earlier than previously thought.

WITHDRAWN

Small Noncoding RNAs: New Paradigms in Plant-Microbe Interactions

The role of small RNAs in host-fungal interactions

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Phytopathology 103(Suppl. 2):S2.187

Small RNAs (sRNAs) are a class of short non-coding regulators that mediate gene silencing in a sequence-specific manner by loading into Argonaute protein (AGO) to target complementary genes. In fungi, although RNAi has been applied as a genetic tool to suppress target gene expression, the natural role of endogenous sRNAs remains enigmatic. Studies in the fission yeast and *Neurospora crassa* revealed functions of sRNAs in genome defense, heterochromatin formation, and gene regulation. However, it is not clear whether sRNAs or RNAi are directly involved in pathogenicity. Genome-wide small RNA profiling identified several small RNAs of *Botrytis cinerea* that can potentially target important regulatory genes in plant hosts, including *Arabidopsis* and tomato. By use genetics, genomics and biochemistry approaches, we demonstrated that some of the *Botrytis*-derived sRNAs can function as effectors to silence host immunity genes by hijacking host small RNA machinery.

The role of viral siRNAs in virus infections of maize

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Phytopathology 103(Suppl. 2):S2.187

RNA silencing is a sequence-specific RNA degradation mechanism that serves as an antiviral defense pathway in plants. Most plant viruses have single stranded RNA genomes and replicate via double stranded RNA (dsRNA) replication intermediates. The viral dsRNA triggers antiviral silencing in the host. It is processed by Dicer-like ribonucleases to produce short interfering RNAs (siRNAs) that incorporate into a RNA-induced silencing complex (RISC). Within RISC, the viral siRNA acts as a guide to

direct the complex to complementary target RNAs, which are then destroyed. In this way, viruses provide the molecular tools (siRNAs) that lead to their own destruction, providing a potent and specific antiviral defense. Here we report an analysis of the population of viral siRNAs that accumulate during infection of maize with three different viruses. In each case, we find that viral siRNAs comprise a large proportion of the total small RNAs in infected cells and that viral siRNAs are generated along both strands of the entire genome. However, the analysis identified a few regions of the viral genome that generated very high levels of siRNAs. The characteristics of these “hotspot” viral siRNAs will be discussed. The data raise the intriguing possibility that these abundant viral siRNAs mediate an additional level of antiviral silencing by targeting host genes that are required for efficient viral replication.

The role of sRNAs in the virulence of the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*

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Phytopathology 103(Suppl. 2):S2.187

Gram-negative γ -proteobacteria of the genus *Xanthomonas* are devastating plant pathogens that infect economically important crop plants. *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) causes bacterial spot disease on pepper and tomato and has been developed as a model plant pathogen. While pathogenicity of *Xcv* depends on the type III secretion (T3S) system which translocates bacterial effector proteins directly into the host cell cytosol the interaction with plants is modulated by additional virulence factors. In a dRNA-seq approach we have identified small RNAs (sRNAs) in *Xcv*, some of which are constitutively expressed and highly abundant, whereas others are co-expressed with the Hrp (T3S) system and type III effectors. sRNAs are typically 80-200 nucleotides long, often display a stable secondary structure and either interfere with translation of bacterial mRNAs or enhance their translation. Recent work was focussed on two selected sRNAs, sX12 and sX13, and RNA-binding proteins from *Xcv* strain 85-10. Genetic studies revealed a contribution of both sRNAs to the outcome of infection, i.e. deletion of the corresponding genes strongly delays the induction of the hypersensitive response in resistant plants. Possible targets of sX13 and the role of the RNA-binding protein Hfq will be discussed.

***Phytophthora* produces RNA-silencing repressors to promote infection**

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Phytopathology 103(Suppl. 2):S2.188

Effectors are essential virulence proteins produced by a broad range of parasites. Upon entry into host cytoplasm, pathogen effectors manipulate specific physiological processes or signaling pathways to subvert host immunity. However, the majority of effectors, especially those produced by eukaryotic pathogens, remain functionally uncharacterized. Recently, we reported that two RXLR effectors from *Phytophthora sojae* can suppress RNA silencing in plants. Furthermore, these *Phytophthora* Suppressors of RNA silencing (PSRs) as well as some Viral Suppressors of RNA silencing (VSRs) promote *Phytophthora* infection. These data demonstrate that *Phytophthora* pathogens have evolved effectors that target host RNA silencing processes for the benefit of disease development. These findings are also consistent with a

role of small RNAs in plant defense against *Phytophthora*. Here, we will report our recent progress on the mechanistic analysis of the RNA silencing suppression activities and virulence functions of PSRs. We will also discuss the role of small RNAs in regulating plant defense during *Phytophthora* infection.

Application of small RNAs and RNA-silencing mechanisms in fungi

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Phytopathology 103(Suppl. 2):S2.188

Like other eukaryotic organisms, fungi produce a wide array of small RNAs. The function of many classes remain unknown. I will present current knowledge of small RNAs in fungi and discuss strategies to exploit RNA-silencing mechanisms to gain new knowledge and develop practical applications.

Virus Intracellular Accumulation and Movement as a Target for Disease Control

Investigating a new role for the *Cauliflower mosaic virus* P6 protein: Delivery of virions to plasmodesmata

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Phytopathology 103(Suppl. 2):S2.188

The P6 protein of *Cauliflower mosaic virus* (CaMV) assembles in the cytoplasm into large, amorphous inclusion bodies (IBs) that associate with and move on microfilaments. The CaMV IBs are considered virion factories, as they are the site for genome amplification and virion assembly. Because the majority of virions are associated with P6 inclusion bodies, we have hypothesized that P6 IBs function to move virus complexes or virions within the cell to the plasmodesmata. A yeast two-hybrid screen of an *Arabidopsis* cDNA library with CaMV P6 as the bait has identified two proteins that might have distinct roles in intracellular trafficking of CaMV. One *Arabidopsis* protein identified in the screen is CHUP1 (Chloroplast Unusual Positioning 1), a protein localized to the outer envelope of chloroplasts and is responsible for their movement on actin microfilaments. Transient co-expression of CHUP1 and P6 tagged with fluorescent proteins revealed that CHUP1 and P6 co-localize within the cell. Furthermore, expression of a truncated CHUP1 blocked the movement of P6 IBs. A second protein, C2CDMT, is a calcium-dependent membrane targeting protein with a C2 domain. Transient co-expression studies with C2CDMT with P6 tagged with fluorescent proteins has revealed that some P6 IBs associate with C2CDMT at plasmodesmata. Taken together, these studies indicate that P6 IBs may be able to utilize host proteins for intracellular movement on microfilaments to travel to plasmodesmata.

Virus-vector-host interactions during movement and transmission of *Grapevine fanleaf virus*

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Phytopathology 103(Suppl. 2):S2.188

Grapevine fanleaf virus (GFLV) is a nepovirus responsible of a severe grapevine degeneration observed in vineyards worldwide. GFLV is specifically transmitted from grape to grape by the ectoparasitic nematode *Xiphinema index*. GFLV moves from cell-to-cell via plasmodesmata as entire virions through viral encoded tubules that result from the self-assembly of the movement protein (MP). Structurally, GFLV is an icosahedral virus of 30 nm of diameter with a pseudo $T = 3$ symmetry composed of 60 identical subunits. In recent studies we have been able to resolve the atomic structure of GFLV and identified surface-exposed structural motifs essential for GFLV transmission and movement. Rods shaped viruses generally accommodate the production of fluorescently labeled viral particles consisting of fluorescent protein (FP)-coat protein (CP) fusions. In contrast, icosahedral viruses such as GFLV are incompatible with such an approach probably due to steric hindrance that totally prevents virion formation and movement. To circumvent these limitations, we produced single-domain antibody fragments also named

Nanobodies that specifically recognize GFLV. When fused to FP and expressed *in planta*, these so-called chromobodies act as biosensors compatible with the spatio-temporal visualization of viral particles during the different steps of the virus life cycle.

The importance of chloroplast interactions for local and systemic movement of some members of the *Alphaflexiviridae*

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Phytopathology 103(Suppl. 2):S2.188

Alternanthera mosaic virus (AltMV; genus *Potexvirus*) and *Lolium latent virus* (LoLV; *Lolavirus*) are distinct members of the family *Alphaflexiviridae*. Each requires interactions with the chloroplast in order to move efficiently cell-to-cell or systemically throughout the plant host. In AltMV, deletion of any one of the Triple Gene Block (TGB) proteins eliminates systemic movement; however, while deletion of either TGB1 or TGB2 restricts replication to the initially infected cell, deletion of TGB3 allows spread to a few adjacent epidermal cells but not movement into the mesophyll. TGB3 is targeted to the chloroplast, where it may serve as an anchor for the viral replicase complex at the chloroplast outer membrane. Alteration of two residues in the N-terminal domain of TGB3 prevents localization to the chloroplast, and disrupts interactions with nuclear-encoded Photosystem II oxygen-evolving complex protein PsbO. The LoLV genome is encapsidated in two carboxy-coterminal coat protein (CP) variants in equimolar proportion, of which the larger contains an additional 48 residues including a 42 residue chloroplast transit peptide (cTP), leading to proteolytic cleavage and an alternative means of production of the smaller CP. Mutational analysis of each of the in-frame CP start codons, and of the proteolytic cleavage site, showed that the N-terminal cTP domain is critical for efficient cell-to-cell and functional systemic movement, but is not required for virus replication.

Host membrane recruitment for replication and intercellular movement of *Turnip mosaic virus*

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Phytopathology 103(Suppl. 2):S2.188

Plant RNA viruses induce in infected cells the formation of elaborate organelle-like platforms that sustain viral RNA synthesis and cell-to-cell movement. Confocal and electron microscopy images show that *Turnip mosaic virus* (TuMV) infection leads to significant rearrangements of the early secretory pathway. Infection is associated with the formation of at least two distinct types of sub-cellular compartments induced by the viral protein 6K₂: a perinuclear globular structure and cortical endoplasmic reticulum (ER)-associated motile vesicular structures. The perinuclear globular structure contains ER, Golgi, and COPII cotomers, along with viral RNA and replication proteins. These structures are not isolated organelles and are dynamically connected to the bulk of non-modified endomembranes. The motile vesicular structures, which also contain viral RNA, are derived from the globular structure and move along transvacuolar ER tubules toward the plasma membrane to become associated with plasmodesmata. Once at plasmodesmata, the vesicles can move over into the neighboring cells leading to their infection. The viral protein 6K₂ has a transmembrane domain (TMD) that is responsible for membrane alterations. The N-terminal soluble tail of the protein has an ER-export signal that is required for cell-to-cell infection. The TMD of 6K₂ also contains a motif that prevents the fusion of 6K₂ vesicles with the plasma membrane on their way to plasmodesmata.

Assessing vacuole trafficking and metabolizing components for their influence on tobamovirus-induced disease

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Phytopathology 103(Suppl. 2):S2.189

Tobacco mosaic virus (TMV) induces hypersensitive cell death (HR) in tobacco plants carrying the N resistance gene and this process can be prevented by silencing expression of a vacuolar protease, Vacuolar Processing Enzyme (VPE). The regulatory mechanism underlying VPE-induced programmed cell death and the importance of the tonoplast for virus accumulation are not understood, but it has been shown that the vacuolar membrane is enriched for TMV replication proteins. Furthermore, it has been shown that the TMV replicase protein is responsible for eliciting HR in N-gene tobacco. Using different domains of TMV 126kDa protein as baits, we utilized a yeast two-hybrid screen with Arabidopsis and *N. benthamiana* cDNA libraries. Two of the host proteins that were shown to interact with the 126kDa protein were VPE and a vacuole pathway SNARE protein associated with membrane targeting. Silencing of either VPE or the SNARE protein in *N.*

benthamiana altered TMV primary lesion size and virus accumulation in inoculated leaves. These and additional studies to be presented have identified the viral protein to which VPE binds and a potential pathway by which the TMV replication proteins traffic to the vacuole membrane.

Summary of virus accumulation and movement findings and their potential application, with Q&A

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Phytopathology 103(Suppl. 2):S2.189

Virus intracellular movement is a prerequisite for disease development. Understanding the basic mechanism(s) by which viruses move within cells allows researchers the ability to develop new methods to control virus-mediated quality and quantity losses in important crops. The preceding presentations in this symposium illustrate the considerable progress made in this area for both DNA- and RNA-based viruses. This session will summarize the findings from the previous talks, identifying similarities and differences in the intracellular transport strategies between viruses. Questions will be taken from the audience for the symposium speakers and this interaction will lead to a general discussion of the future of this research for practical application.

Professionalism/Outreach

An Unconventional Classroom: Reaching New Students with Online and Distance Courses and Programs

Selection and use of technology for offering a distance course in plant pathology

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Phytopathology 103(Suppl. 2):S2.189

Demand for effective distance education (DE) offerings is increasing rapidly at all levels of education. However, strained budgets and ever-increasing demands on your time are often viewed as primary constraints in the development of high-quality DE courses. The selection and use of the most appropriate technology can greatly aid in meeting the demands for web-based learning materials while keeping within your budget and still allowing you to do the rest of your job. This talk will emphasize those tools best suited for producing and delivering material in a DE Plant Pathology course as well as short course or other extension based materials.

The challenges and opportunities of teaching plant pathology at a distance

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Phytopathology 103(Suppl. 2):S2.189

Teaching agricultural sciences in an online format at either the undergraduate or graduate level remains a somewhat controversial topic for the Land Grant University. Lab-based courses, such as basic plant pathology, present the greatest challenges when attempting to transfer the full course experience at a distance. However, the reward comes when the course reaches an underserved audience who may not have the opportunity to participate in plant pathology coursework otherwise. Different methods of delivery have been tried in online education from using videoconference technology to share lectures between campuses within a system to the totally online, asynchronous experience that can reach a wider audience. An administrative support system is essential for distance education success in any format. This presentation will discuss the speaker's experience with teaching plant pathology coursework online using different delivery systems over the last 12 years. Additional models for delivering content will be discussed including the system recently developed at the University of Georgia as well as examples of distance education in the agricultural sciences at other institutions.

Teaching tropical plant pathology to a global audience

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Phytopathology 103(Suppl. 2):S2.189

The Global Plant Health (GPH) program funded by the International Research Experience for Students (IRES) program at NSF includes the study of tropical plant pathology and promotes discovery research using hands-on

training in the tropics. A diverse group of undergraduate and graduate student scholars take a course in tropical plant pathology in the spring semester that precedes a week-long field class that includes learning experiences in cacao, banana, pineapple, tropical root crops, floriculture and sugarcane fields. Additional research experiences are provided to GPH interns who work with paired mentors over a six week period in the summer in Costa Rica and the following fall semester at NC State University on research projects on tropical plant diseases. The research projects have direct benefits to society and include opportunities for students to solve real world problems by conducting ecologically-based research in the tropics on economically important disease problems on tropical crops. The tropical plant pathology class is also offered as a distance education class for students outside North Carolina. Students write research reports, and produce a voice over power point presentation of their data to a broad community that includes the Universidad de Costa Rica, an undergraduate student symposium at NC State, and major industry groups including Dole Foods. The transformative IRES in tropical plant pathology enhances food security in both countries.

Masters-level agricultural biosecurity education for location-bound adult learners

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Phytopathology 103(Suppl. 2):S2.189

Online education presents an invaluable opportunity to location bound adult learners seeking to extend their education. Penn State offers an online Master of Professional Studies in Homeland Security with four options including the option in Agricultural Biosecurity and Food Defense. Completion of the four core courses within the option earns a Graduate Certificate in Agricultural Biosecurity and Food Defense. Almost all students enrolled in these programs are working adults and include active duty, deployed and retired military. Many military students are preparing for post-military careers. The online nature of these programs make them available to students who are unable to physically attend a classroom-based program. Previous knowledge of the agriculture food system is low amongst enrolled students however this lack of knowledge does not prevent students from successful completion of the courses. The first of the four core courses is open to students in the other options of the Master of Professional Studies in Homeland Security. One outcome of enrollment in this course is an increase in knowledge of the agriculture food system, the importance of plant and animal biosecurity, and of related issues such as invasive species amongst an audience with little other exposure to such topics. Student response to the course is largely positive with many expressing surprise upon learning about the magnitude and complexity of the agriculture food system.

What's in it for us? Creating financial and academic incentives for faculty in an online degree program

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Phytopathology 103(Suppl. 2):S2.189

There is increased interest in distance education at many institutions of higher learning, in order to reach potential students who find it difficult to come to a main campus for the period needed to obtain a higher degree. The College of Agriculture and Life Sciences at Virginia Tech has an online masters degree program, providing a Masters in Agricultural and Life Sciences. Courses have been taught in this program since 2007. Faculty are very diverse in their initial interest in virtual teaching and in taking up

responsibilities in addition to existing duties. In order to recruit faculty for online course development and in advising of virtual graduate students, the must be transparent discussion on work load involved and the benefits that can accrue with involvement in the program. This presentation will discuss incentives for faculty involvement, including student advising and financial support for teaching efforts.

Counting Beans & Tooting Horns: Effective Metrics for Documenting the Impact of Research and Extension

Introduction—Defining impact: From website hits to change in practice

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Phytopathology 103(Suppl. 2):S2.190

This introductory presentation will launch a special symposium entitled “Counting Beans & Tooting Horns: Effective Metrics for Documenting the Impact of Research and Extension” coordinated by The American Phytopathological Society Extension Committee and supported by the Early Career Professionals and the Plant Pathogen and Disease Detection Committees. The symposium will highlight the value of effective metrics for documenting impact in plant pathology. Programs dependent upon financial support from scarce public resources and other aid donors need to justify the investment both in the short and long terms. Accountability for past funding enhances ability to attract new funding and builds awareness of the quality of the work done to ensure political support and sustainability of national priorities. While many short term impact assessments in agricultural research include “bean counts” or numbers of program attendees, website visits, or grower contact points, accessing the impact of the work through change in practice or status improvement over time and stakeholder groups is much more elusive. Determination of appropriate value metrics (economic, environmental, social) and approaches to valuing impact (ex-post surveys vs. ex-ante extrapolations) is specific to the nature of a program and can be aided by evaluation specialists. Examples of successful impact evaluations in research and extension programs will be shared and discussed in this session.

Impact evaluation: It’s by design

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Phytopathology 103(Suppl. 2):S2.190

The demand for impact evaluation is growing, yet where do you begin? The answer is in a program’s design. The increasing demand for impact evaluation reflects an increasing expectation that programs generate impacts. Yet the logic that runs between a program and its intended impact is often sketchy, making impact evaluation a challenge. This presentation will discuss what impact evaluation is, when it is appropriate, and the basics on how to frame up an impact evaluation. Attendees will improve their knowledge of how to write an integrated, logical, impact program and evaluation plan proposal and learn what goes into designing outcome and impact evaluations.

The importance of documenting impact—A Washington perspective

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Phytopathology 103(Suppl. 2):S2.190

In the past several years, Congress has increased their expectations for accountability. Reporting plays a critical role in future funding and the ability to advance the science agenda in the U.S. NIFA faces frequent inquiries from Congressional offices and the Government Accountability Office (GAO) about progress on projects, spending rates and duplicative funding. There are several strategies to follow that can help address the concerns. For example, ask yourself if you are keeping up your end of the contract. Do you report on a regular basis and by the deadline? Do you know the reporting deadlines? Do you spend your funds? Perhaps the most important factor is HOW and WHAT you report from your project. Often you will hear the terms impact and outcome thrown around randomly. These terms do not refer to results. Results

are progress on a project. Think of impact as being more like conclusions. Results with value added. Even more than that, the impact addresses the most important question a policymaker might ask, “So what?” Recognizing that certain “So what” elements carry more weight than others is important. Economic and human health impacts carry considerable influence. Environmental impacts can also be valuable if reported in a way that speaks to the hearer. The outcomes we seek and the metrics we use, combined with our diligence will determine our success. The quality of our reporting is the one thing we can control about the perception of our research programs.

A case study in documenting impact in research

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Phytopathology 103(Suppl. 2):S2.190

From small grower-funded projects to large multi-state initiatives, assessing impact is critical. Most scientists are well versed in articulating goals, objectives and hypotheses but were never trained to write in terms of outputs, outcomes, impacts or logic models. Documenting the impact of a research project can be challenging particularly since it may take many years for a study to be completed and applied to a field situation. Pathogen population surveys, which can be part of larger epidemiology studies, are one area of research that can provide information to growers and have a noticeable impact within a relatively short period of time. My lab has performed pathogen surveys, using DNA fingerprint methods to type isolates from fairly small-scale outbreaks of bacterial canker of tomato and black rot of cabbage over several seasons. Results from these projects were immediately available to growers with a 95-100% modification of practices based on results. We have also completed a state-wide survey of the Phytophthora blight pathogen with variable impact on grower practices and economic outcomes. Lastly, current research as part of a large multi-state project is focusing on reducing losses to late blight on tomato and potato. We are working to improve methods used to assess and document impact of grower communication, use of a decision support system, and the overall economic impact of the project.

Documenting the impact of 10 years of IPM on Wisconsin cranberry production: A case study in documenting impact in extension

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Phytopathology 103(Suppl. 2):S2.190

Food supply chain partners have expressed growing interest in quantifying sustainability advancements behind the farm gate. This interest includes significant effort to document participatory applied research, educational outreach and implementation of integrated pest management (IPM) strategies and thus has created an opportunity to communicate the important role of extension to diverse audiences. In 2009, for example, the Wisconsin cranberry growers and the University of Wisconsin (UW)-Extension initiated a project to develop a producer-led, research-based cranberry sustainability assessment survey to document advances in social, economic and environmental sustainability parameters over time, including adoption of IPM practices. In a 2012 survey, over 80% of producers scouted crops for plant diseases, 88% managed water to minimize disease risk and 92% sampled soil and plant tissue nutrient status according to research-based recommendations – all practices developed through applied land-grant research and adopted through educational efforts by UW-Extension. Benefits for extension programs from these efforts included: the process of developing assessments has strongly linked research and outreach personnel with agricultural producers; gaps in knowledge or practice identified in the assessments were used to leverage research funding; and, the advancements in agricultural production based on applied research and public outreach were communicated broadly.

Plant Pathologists of the Future: Showcasing the Top Graduate Students from APS Division Meetings

Prevalent citrus diseases in Puerto Rico

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Phytopathology 103(Suppl. 2):S2.191

Citrus diseases have destroyed millions of hectares in the world. There are diseases affecting citrus in Puerto Rico, that are causing losses without being detected or identified. A survey was conducted from February 2011 to February 2012 in the major citrus producing areas of the island for detection of citrus greening (CG) and other diseases. Fifteen nurseries and seven commercial orchards located in 20 municipalities were sampled. Symptomatic plants were collected and processed at the Plant Disease Clinic in Juana Diaz. Diagnosis of fungal diseases was carried out by isolation of disease tissue in artificial media, ELISA test for *Citrus tristeza virus* (CTV) and citrus variegated chlorosis (CVC). CG was identified using Polymerase Chain Reaction (PCR) with specific primers (OI1, OI2c). A total of 330 samples were processed and the major fungal diseases identified were anthracnose caused by *C. gloeosporioides* and fruit spot by *Alternaria* spp. CG by '*Candidatus Liberibacter asiaticus*' and CTV were detected in 6% and 14% of the samples, respectively. The introduction of propagative material and fruit imports from other countries represents a threat for exotic diseases entering inadvertently to Puerto Rico. Regular screening in commercial orchards of pathogens of quarantine importance, such as black spot and citrus canker, will be implemented to protect citrus production in the island.

Detection of boscalid resistance and the H272R mutation in the *SdhB* gene of *Blumeriella jaapii*

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Phytopathology 103(Suppl. 2):S2.191

Cherry leaf spot (CLS), caused by the fungus *Blumeriella jaapii*, is a major disease of tart cherry (*Prunus cerasus*) trees, leading to early defoliation which results in poor fruit quality, reduced fruit set, and death of the tree. Pristine, a commonly-utilized fungicide for CLS management in Michigan, is a premix of boscalid, a succinate dehydrogenase inhibitor, and pyraclostrobin, a quinone outside inhibitor. Reduced efficacy of Pristine for CLS control observed in field trials and commercial orchards highlighted the importance of resistance monitoring. A total of 1,288 isolates from commercial orchards and 111 isolates from non-treated trees were collected in 2010 and 2011 and assayed on boscalid-amended media at concentrations of 0, 0.1, 0.5, 1, 2.5, 5, 10, and 25 µg/ml. The minimum inhibitory concentration (MIC) of boscalid was determined after incubation at 23 C for 14 days. Isolates from non-treated trees had MIC values ranging from 0.1 to 0.5 µg/ml while isolates from commercial orchards ranged from 0.1 to >25 µg/ml. Isolates with MIC values of >25 µg/ml were considered resistant and comprised 22% and 35% of isolates in 2010 and 2011 respectively. Sequencing of the *SdhB* gene of resistant isolates led to the detection of the amino acid mutation H272R known to confer boscalid resistance. The occurrence of the H272R mutation in Michigan populations of *B. jaapii* is correlated with the reduction in sensitivity to boscalid observed in commercial orchards.

Northeastern Division: Detection of *Peronospora variabilis* in quinoa seeds

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Phytopathology 103(Suppl. 2):S2.191

Quinoa (*Chenopodium quinoa*) is the most recent Andean crop to have global impact, but research on management of quinoa diseases is insufficient. Quinoa downy mildew, caused by *Peronospora variabilis* (formerly *Peronospora farinosa* f. sp. *chenopodii*), is the key disease of quinoa and can lead to severe yield losses. *P. variabilis* oospores can be seedborne and previous seed detection methods have relied on visual detection and seedling assays. However, these methods are time-consuming, prohibiting the screening of large numbers of seed. In this research, molecular methods were developed to detect *P. variabilis* in quinoa seeds. Thirty-three seed lots of domestic and imported quinoa, along with seed of *Chenopodium pallidicaule* and *Chenopodium album*, were analyzed using two PCR based detection methods. By using a sequencing-based detection method, *P. variabilis* was detected in 32 of 33 seed lots. A method that employed PCR with *P. variabilis* specific primers detected the pathogen in 31 of 33 seed lots. *P. variabilis* was never

detected in *C. pallidicaule* or *C. album* seed lots. Seedborne oospores of *P. variabilis* likely contribute to downy mildew epidemics in Andean countries and have allowed the pathogen to spread to countries beyond South America. This research allows for rapid, high throughput detection of *P. variabilis* in quinoa seeds, which is the first step toward creating certified *P. variabilis*-free quinoa seed.

Dualism in symbiosis: Growth and defense enhancement of symptomless infection by the pathogen *Fusarium circinatum* in *Pinus radiata* seedlings

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Phytopathology 103(Suppl. 2):S2.191

Many fungal symbionts can inhabit their hosts both as endophytes that cause no symptoms and as pathogens, but their activity as pathogens generally receives the most attention. *Fusarium circinatum* is a pine symbiont that can cause a disease known as pitch canker but it can also inhabit pine tissue (typically roots) without inducing symptoms. Studies were undertaken to characterize physiological effects of this asymptomatic association in *Pinus radiata* seedlings. Seedlings sustaining symptomless infections had a total shoot and root fresh weight equal to or greater than un-infected plants, when measured at both 12 and 40 weeks after planting. This was accompanied by alterations in root system architecture, with greater branch density in infected plants. Symptomless infections enhanced seedling resistance to later above ground infections, reducing stem lesion size and increasing survival frequency. Field surveys documented symptomless infections of seedlings in native stands of *P. radiata*, with up to 80% of two-year old healthy-looking seedlings harboring root infections. These results suggest that in addition to being a cause of disease, *F. circinatum* can have a commensal relationship with *P. radiata*, and may also promote seedling survival in native stands, under some circumstances. As a consequence of these diverse effects on fitness, selection for genetic resistance to disease caused by *F. circinatum* may be less intense than would otherwise be expected.

Xylella fastidiosa phoP/Q two-component system mediates colonization of grapevines and may be a potential target for Pierce's disease control

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Phytopathology 103(Suppl. 2):S2.191

Xylella fastidiosa (*Xf*) is a gram-negative, xylem-limited plant pathogenic bacterium that causes disease in a variety of economically important agricultural crops including Pierce's disease of grapevine. *Xf* biofilms formed in the xylem vessels of plants play a key role in early colonization and pathogenicity by providing a protected niche and enhanced cell survival. Biofilm formation is induced by the process of quorum sensing and may be mediated by two-component regulatory systems. Like many other bacteria, *Xf* possesses homologs to the two component regulatory system PhoP/Q which differentially regulates genes in responses to divalent periplasmic cation concentration and other environmental stimuli. *Xf* knockout mutants deficient in the production of PhoP and PhoQ exhibit phenotypic differences in cell dispersal and clumping when grown in liquid culture. *Xf* phoP/Q mutants had a 42% and 47% reduction in biofilm formation, and a 42% and 36% reduction in cell-cell aggregation, respectively. Grapevine pathogenicity assays showed phoP/Q mutants are non-pathogenic and are unable to successfully colonize or move within the xylem vessels. These results may be due to the inability of *Xf* to successfully sense, respond and adapt to the nutrient-limited environment of the xylem. This regulatory system, essential for *Xf* to cause Pierce's disease, may be a novel target for disease control through anti-PhoP/Q peptides.

Phytophthora cinnamomi as a possible contributor to white oak (*Quercus alba*) decline in Mid-Atlantic forests

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Phytopathology 103(Suppl. 2):S2.191

To evaluate the association of *Phytophthora cinnamomi* with white oak (*Quercus alba*) decline in Mid-Atlantic U.S. forests, 193 healthy white oaks, 247 declining white oaks, and 182 other plants were sampled at 102 sites between 2011 and 2012. At each site, soil and roots from white oaks and soil from other hosts were collected. *Phytophthora* species were isolated using a baiting method and *P. cinnamomi* soil inoculum level was quantified using a wet-sieving method. White oak roots were scanned to measure total fine root lengths. *Phytophthora* was isolated from 42% of the sites. *P. cinnamomi* was isolated most commonly, from 60% of positive soils. Over five other species were isolated infrequently, from the remaining 40% of positive soils. *P. cinnamomi*'s range was restricted to the southern part of the study region in USDA hardiness zones 6 and 7, and not found further north in zone 5. Soils

from zone 7 (warmer) had twice as much inoculum as zone 6. Soils from white oaks compared to 16 other hosts did not have significantly different inoculum levels. White oak fine root amounts differed significantly between sampling years and hardiness zones. In both years, fine root amounts were significantly lower from *P. cinnamomi*-infested trees vs. non-infested trees, except in zone 7. Our data suggests that *P. cinnamomi* influences root health of white oaks mainly in the southern Mid-Atlantic region and may pose a threat to forests further north if warming trends continue.

Genetic analyses of *ntpR* encoding a novel negative regulator for toxoflavin production in the rice-pathogenic bacterium *Burkholderia glumae*

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Phytopathology 103(Suppl. 2):S2.192

Burkholderia glumae is the major causal agent of bacterial panicle blight of rice, a disease that can cause severe yield losses, and is becoming an

increasingly important pathogen worldwide. The phytotoxin, toxoflavin, is the most important virulence factor of *B. glumae* and is required for full virulence. Transposon mutagenesis of a virulent strain of *B. glumae* yielded a number of mutant derivatives with increased toxoflavin production. A few of these mutant derivatives were found to be disrupted in a putative open reading frame (ORF) encoding a LysR-type transcriptional regulatory protein. This putative ORF was named *ntpR* for negative regulator of toxoflavin production LysR. Additional independent mutations of *ntpR* created via homologous recombination reproduced the phenotype of the original mutant derivatives. Complementation of the *ntpR* mutant derivatives with an *ntpR* clone resulted in a substantial reduction in toxoflavin production, indicating that *ntpR* is a negative regulatory gene for toxoflavin production. In order to develop a better picture of the regulatory functions of *ntpR* in *B. glumae*, transcriptome profiles and virulence gene expression associated with *ntpR* are currently being analyzed. Increased knowledge of *B. glumae* virulence factors and their regulatory mechanisms will contribute to a greater understanding of bacterial pathogenesis by this pathogen and will help to develop effective disease control strategies.