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Abstracts of Special Session Presentations

Biology of Pathogens

International Perspective on Fusarium Head Blight

A toxic character: *Fusarium graminearum* and mycotoxin biosynthesis

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Trichothecene mycotoxins produced during infection of wheat and barley by *Fusarium graminearum*, can contaminate the grains and thus render the crop unusable. Trichothecenes also may act as virulence factors that intensify the disease damage (*viz.* necrosis and blighting) done to the grain during infection. In the past five years, much has been learned about the chemical and structural triggers of trichothecene biosynthesis during plant infection. The genome sequence of the fungus also has yielded much information on the genetic adaptations and developmental events responsible for toxin synthesis. Structural changes to fungal cells that produce trichothecenes on plants and under toxin-inducing conditions *in vitro* point to morphological requirements of toxigenic hyphae. Cellular mechanisms for toxin export in the fungus are still largely unknown. The current status of trichothecene biosynthesis, regulation and transport will be summarized.

Mycotoxin production during infection of cereals

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The fungal pathogen *Fusarium graminearum* is the causal agent of Fusarium head blight (FHB) of small grain cereals and cob rot disease of maize. During infection, the fungus produces mycotoxins, like the trichothecenes deoxynivalenol (DON) and nivalenol (NIV). The key enzyme in their biosynthesis is the trichodiene synthase (Tri5). Disruption of the Tri5 gene restricts the infection to the inoculated wheat spikelet, independent of the chemotype (NIV or DON) and the trichothecene amount originally produced by the wild type strain. Surprisingly, trichothecenes are not essential for barley or maize colonization in greenhouse experiments. We recently cloned a reporter strain with the Tri5 promoter fused to eGFP. Monitoring mycotoxin induction during the infection process we showed a time dependent induction during infection structure development and a tissue specific production during wheat infection. Especially the developing kernel and the rachis node of wheat induce DON production by *F. graminearum*. In vitro, several different DON

inducing conditions are described, among them oxidative stress, low pH and different nitrogen sources like ammonium rich amino acids and polyamines like putrescine. We will provide a model of the DON inducing conditions during wheat infection combining the above described inducers.

Identification of candidate genes for head blight and deoxynivalenol resistance

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We are investigating the mechanisms underpinning host resistance to deoxynivalenol (DON) and Fusarium diseases of wheat and barley. Using functional genomics, we identified genes associated with DON responsiveness and DON resistance. Several such genes, including a multidrug ABC transporter, directly contribute to DON resistance; attenuating this gene through virus-induced gene silencing increased the susceptibility to DON. *Pseudomonas fluorescens* strain MKB158 was identified as a bacterium that reduces FHB levels, associated toxin accumulation and yield losses. Based on transcriptomic, biochemical and phenotypic studies, we deduced and confirmed that auxin is involved in bacterial priming of host resistance. Studies on another plant hormone, brassinosteroid, identified the receptor Br1 as a key regulator of FHB resistance and toxin build-up in grain. On the basis of microarray analysis, we identified genes and pathways differentially regulated in a Br1 mutant as compared to wild type barley. On the basis of all our studies, we can conclude that there is a biochemically diverse array of genes that can be targeted to control FHB, thus offering great scope for breeding genotypes with effective long-term resistance to FHB. However, in the context of a practical breeding programme, the effect of some such genes on other biotic and abiotic interactions must now be investigated.

The role of trichothecenes in the Triticeae-*Fusarium graminearum* interactions

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Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, is a major disease problem for the small grain crops wheat and barley. During infection, *F. graminearum* produces trichothecene mycotoxins such as deoxynivalenol (DON) that increase the aggressiveness of the fungus and reduces grain quality. Thus, we are interested in identifying genes that protect wheat and

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barley from the toxic effects of trichothecenes. Previous work had identified an *Arabidopsis* DOGT1 gene, encoding an UDP-glucosyltransferase (UGT), as involved in trichothecene resistance via conjugation of DON to DON-3-glucoside (D3G). We examined transcript profiles in wheat and barley during *Fusarium graminearum* infection and inoculation with the trichothecene deoxynivalenol (DON). From these experiments, we identified a set of 10 barley UDP-glucosyltransferases (UGT) that were upregulated during *F. graminearum* infection or DON treatment. We screened this set of UGTs in yeast and identified a barley UGT (HvUGT13248) gene that conferred resistance to DON via conjugation to D3G. Transgenic *Arabidopsis* overexpressing HvUGT13248 exhibited the ability to grow on media containing DON. DON feeding studies on the transgenic *Arabidopsis* showed that DON was conjugated to D3G. In contrast to prior work in *Arabidopsis* overexpression DOGT1 exhibited a dwarf phenotype due to brassinosteroid conjugation, transgenic *Arabidopsis* overexpressing HvUGT13248 did not exhibit dramatic changes in morphology and did not conjugate the brassinosteroid castasterone. More recently, we developed transgenic wheat

overexpressing HvUGT13248 and showed that these lines exhibited high type II FHB resistance.

Host colonization leading to sporulation in *Fusarium graminearum*

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Fusarium graminearum is unique among the major *Fusarium* pathogens of grains, in that it is sexually prolific. There is strong evidence that for *F. graminearum*, sexual spores are important dispersal agents. Infection and colonization processes for some grain crops predetermines the relative placement and quantity of sexual fruiting bodies that appear in infected plants, provided that environmental conditions are conducive. We will present our findings on the host-pathogen interactions that lead to perithecia production in infected host tissue, and discuss differences in the process as they are affected by host anatomy and maturity.

New Insights into the Virulence Mechanism of Plant-Pathogenic Bacteria

The role of the type III secretion system in necrotic pathogens

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Necrotic pathogens prosper when plant cells die and therefore may induce programmed plant cell death during pathogenesis rather than inhibiting it by interfering with apoptotic pathways, as biotrophic or hemibiotrophic pathogens do. The necrotic soft rot pathogen *Pectobacterium* causes disease on diverse plant species and examination of its type III secretion system (T3SS) supports a model where necrotic pathogens prosper by inducing plant cell death. *Pectobacterium* and the related genus *Dickeya* appear to encode, at most, only a single type III effector. Some strains in these genera lack T3SS entirely. Deletion of the single effector, DspE, eliminates the ability of *Pectobacterium* to elicit a hypersensitive response on solanaceous plants, but does not greatly impair pathogenesis on plant stems or tubers. Unlike the hemibiotrophic pathogens *Pseudomonas syringae*, which encodes numerous type III effectors, the *Pectobacterium* T3SS appears to have little impact on plant gene regulation early in pathogenesis. DspE homologs encoded by soft rot Enterobacteriaceae pathogens are smaller than homologs encoded by hemibiotrophs. However, WXXXD/E motifs required for DspE activity in hemibiotrophs are conserved in necrotroph DspE homologs and these motifs are required for activity of the *Pectobacterium* DspE homolog.

Insights into the virulence mechanism of *Xanthomonas citri* subsp. *citri*

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The genus *Xanthomonas* is an important group of Gram-negative plant pathogenic bacteria, which infects approximately 124 monocotyledonous and 268 dicotyledonous plants. The genus, *Xanthomonas*, has become an important model organism for studying plant-microbe interactions and for understanding bacterial pathogenicity and virulence mechanisms. Among the diseases caused by members of the genus *Xanthomonas*, citrus canker is one of the most serious diseases of most commercial citrus cultivars resulting in significant losses worldwide. This devastating disease is caused by *Xanthomonas citri* subsp. *citri*. *X. citri* subsp. *citri* is spread primarily by rain splash; it enters its hosts through wounds and natural openings such as stomata and forms distinctive raised, necrotic lesions surrounded by oily, water-soaked margins and yellow chlorotic rings on leaves, stems, and fruit. Eventually, the epidermis ruptures, and massive numbers of bacterial cells emerge to the plant surface, where the bacteria are readily available for rain splash and repeated infection cycles. To understand the virulence mechanisms of *X. citri* subsp. *citri* underneath the infection cycle, we conducted genomewide microarray and RNA-Seq analyses to characterize the critical regulators including HrpX, HrpG, RpfG, and RpfC, which are critical for the pathogenicity of *X. citri* subsp. *citri*. How *X. citri* subsp. *citri* coordinates its diverse virulence factors will be presented. Genome wide mutagenesis was used to further characterize the genetic determinants of the virulence mechanism of *X. citri* subsp. *citri*. Several novel virulence factors of *X. citri* subsp. *citri* will be presented.

Ooze and rots: How enteric plant pathogens utilize cyclic di-GMP, small RNAs, and quorum sensing to regulate major virulence genes

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We have been studying the regulation of critical virulence determinants in the enteric plant pathogens *Dickeya dadantii* and *Erwinia amylovora*. Overexpression of either of two *E. amylovora* GGDEF proteins resulted in increased intracellular cyclic di-GMP levels, amylovan synthesis, and biofilm formation. The Hfq small RNA chaperone was shown to be an important virulence factor and positively controlled type three effector translocation. A total of 10 putative small RNAs were identified in the *E. amylovora* genome, and two of these (RyhA and RprA) were found to positively regulate the expression of the type three effector DspE. In *D. dadantii*, we demonstrated that arabinonuclease PNPase and regulatory small RNA RsmB function in the post-transcriptional regulation of the type III secretion system. A reduced degradation of *hrpL* mRNA was observed in *Dpn* compared to the wild type. *Dpn* also showed an RsmB transcript pattern different from the wild type, with additional shorter RsmB transcripts truncated at the 3' end present along with the full length RsmB as observed in the wild type. Functional analysis later showed that the RsmB transcripts with different nucleotides truncated at 3' end still retain some biological functions in the post-transcriptional control of *hrpL* mRNA. Our genetic and mechanistic studies contribute to our goal of an ultimate dissection of the roles of second messenger molecules and post-transcriptional regulation in enteric bacterial pathogenesis.

The role of the cell surface lipopolysaccharide molecule in *Xylella fastidiosa* biofilm formation and virulence in the grapevine host

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Xylella fastidiosa (*Xf*) is a Gram-negative, xylem-limited bacterium that causes disease on several hosts, such as grapevine, almond, and oleander. Lipopolysaccharide (LPS) comprises approximately 70% of the outer membrane of Gram-negative bacteria, and because it is largely displayed on the cell surface, it mediates interactions between the bacterial cell and its surrounding environment. LPS is comprised of a conserved lipid A-core oligosaccharide component and a variable O-antigen portion. We are investigating the contribution of the O-antigen to *Xf* surface attachment and biofilm maturation: two critical steps for successful infection of the host xylem. Moreover, LPS has been implicated as a major virulence factor for several bacterial pathogens and we are exploring the role of LPS as a virulence determinant for *Xf*. By targeting key genes involved in O-antigen biosynthesis, we have demonstrated that a fully formed O-antigen moiety is a critical virulence factor for Pierce's disease development in grape and that depletion of the O-antigen compromises the ability of *Xf* to colonize the xylem. The O-antigen has also been implicated in host specificity for mammalian pathogens. The wide host range, yet stringent host specificity of different *Xf* subspecies affords an opportunity to study the molecular mechanisms underlying host specificity in a phyto-bacterial pathosystem.

The global regulatory network for the virulence of *Burkholderia glumae*, the major causal agent of bacterial panicle blight of rice

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Burkholderia glumae causes bacterial panicle blight of rice, which is an emerging rice disease problem in many rice-growing regions around the world including the southern United States. Production of the phytotoxin, toxoflavin, and lipase and biogenesis of flagella are crucial for the virulence of *B. glumae*. These virulence-related phenotypes are globally regulated by the *N*-acyl homoserine lactone (AHL) quorum-sensing system of *B. glumae*, which is conferred by *tofl* and *tofR* genes encoding an AHL synthase and an AHL receptor, respectively. According to our recent study, *PidS/PidR* two-component system controls a functional type III secretion system and toxoflavin production in *B. glumae*. In this study, additional new regulatory factors of *B. glumae* were identified through various mutational approaches and characterized in terms of their functions in virulence-related phenotypes. Among the new regulatory genes, *ntrR* encoding a LysR-family protein and *teprR* encoding an RpoN-dependent response regulator acted as negative regulators for toxoflavin biosynthesis and virulence. *tofM* encoding an RsaM homolog and located between *tofl* and *tofR* was also characterized as a new regulatory gene for toxoflavin production in this study. Further functional and genetic studies on these newly found regulatory components in conjunction with other known regulatory systems, such as the *TofI/TofR* AHL quorum-sensing system, would provide a better view on the global regulatory network of this pathogen for pathogenesis.

How high-throughput sequencing technology helps our understanding of plant-pathogenic bacteria (overview)

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Draft genome sequencing has become cost-effective and time saving with the advent of next generation sequencing methods. The increasing scale of genomics provides a rapid means for identifying virulence factors and for generating new hypotheses to explain the complexities of host-pathogen interactions. Comparative genomics has raised a number of questions as to how diverse species evolve to extend host range and as to the role of type III effectors and molecular mechanisms in driving the evolution of the pathogen. In Florida, the bacterial spot of tomato pathogen *Xanthomonas euvesicatoria* (*Xe*) was the only species associated with tomato. In 1991, *Xanthomonas perforans* (*Xp*) was identified and as of 2006 has become the predominant species in tomato fields. Until recently, only *Xe* has been pathogenic on pepper. *AvrXv3* present in *Xp* strains restricted host range on pepper. We recently isolated and sequenced an *Xp* strain that is able to infect pepper. We also sequenced *Xp* strains representative of the four tomato growing regions in Florida. *In silico* analysis of the genes present in the new pepper *Xp* strain, but absent from the previously sequenced *Xp*91-118, revealed candidate virulence factors on pepper. Differences in effector suites have evolved in these closely related field strains. Phylogenetic analysis based on MLST and SNPs revealed diversity among these field strains and, when related to the geographical distribution, possible clues to their evolution.

Potato virus Y—An Old Virus and a New Problem in Potato

PVY as an emerging potato problem in North America

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In the past decade, *Potato virus Y* (PVY) has re-emerged as a significant disease affecting seed potato production and is threatening to become a tuber quality issue. The resurgence of PVY is due, in part, to widespread acceptance of potato varieties that express mild or no foliar PVY symptoms, to displacement of the ordinary PVY strain by recombinant strains that often induce mild foliar symptoms and tuber necrosis, and to increased late season spread of the virus due to changes in aphid populations. These late season infections often are not manifested in foliar symptoms, but result in infected tubers. Each of these changes hampers the detection and removal of PVY-infected plants during field inspections and rouging operations, and leads to more virus being present in seed stocks. A national PVY project, outlined in this talk, has partnered with seed certification and regulatory agencies, and grower organizations across the U.S. to address the needs of the potato industry. Our immediate goal is to work with seed certification agencies to reduce the level of virus in seed stocks below economic thresholds and to eliminate seed lots harboring the tuber necrotic strain. The longer term goals are to improve on-farm Best Management Plans to reduce the impacts of PVY on farm profitability and product quality. Project outcomes are translated to stakeholders via www.potatovirus.com that includes an open discussion forum on any aspect of PVY or other potato virus diseases.

Breeding potato for PVY resistance

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In recent years, *Potato virus Y* (PVY) has emerged as a serious disease problem in potato production areas throughout North America. One explanation for this increase in incidence is that some widely planted cultivars express mild or no symptoms, preventing effective rouging of infected plants. Furthermore, recent investigations have revealed a significant increase in the proportion of PVY recombinants (PVY^{N:O}) in major production areas. Effective strategies to address this emerging problem are lacking. The development of cultivars with improved levels of PVY resistance would provide one such strategy. We have identified a new source of extreme

resistance to PVY^O and PVY^{N:O} in the diploid wild potato relative *Solanum chacoense*. All plants in accession 275138 were resistant to PVY, while variability for resistance was found in accession 320285. Segregation ratios in the F1 and backcross populations indicate that PVY resistance is conferred by a single dominant gene. The 8,303 SNP array developed by the SolCAP initiative is being used to map the gene in a diploid hybrid population created by crossing a heterozygous resistant clone to a susceptible wild species clone (*S. tarijense*, PI558129). In addition, a diploid adapted fertile clone has been selected from a cross between a homozygous resistant *S. chacoense* clone and a susceptible cultivated *S. tuberosum* clone. It expresses genes for the production of numerically unreduced pollen and eggs, so when crossed to tetraploid cultivars, it produces tetraploid offspring carrying the resistance gene. In addition, it carries the dominant allele of a gene that overcomes the gametophytic self-incompatibility system, so it can be self-pollinated to create clones homozygous for the PVY resistance gene.

Modeling aphid vector flights and improved control of *Potato virus Y*

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The US seed potato industry has identified PVY as its most serious disease problem and is looking for ways to reduce overall disease incidence below acceptable levels. In Wisconsin, seed potato crops are threatened by infection of PVY, vectored by several species of potato colonizing and non-colonizing species. The dispersal and movement of PVY vectors are influenced by processes operating across different temporal and spatial scales (e.g. climate, weather, or host plant condition). We describe a modeling framework that uses a generalized linear mixed modeling approach to partition the variance associated with aphid capture data. These approaches were applied to long term, multi-site and multi-year, aphid suction trap data sets from the North Central Region, Aphid Suction Trap Network. Variance estimates of aphid abundance among years significantly exceeded similar estimates among locations (regions). This suggests that factors such as season better explain variation in aphid abundance than factors driving differences among locations (e.g. surrounding habitat characteristics). These findings are consistent with the hypothesis that selected aphid species are migrating into fields from moderate to long distances. Taken together, this approach improves our understanding of the patterns of variation in aphid abundance, thereby increasing our knowledge of when the aphid vectors move into susceptible seed potato fields and when they spread the virus to highly susceptible varieties. A more accurate assessment of the phenology of the principle aphid vector flights provides an opportunity to deploy behavioral modifying crop protectants at critical periods to limit the transmission of PVY to potato.

PVY and Canadian experience

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Potato virus Y (PVY) is an evolving problem throughout the world and Canada is no exception. PVY made headlines in Canada in 1990s when PVY^N was discovered in Eastern Canada, which resulted in the loss of export markets. Since then significant progress has been made in PVY research. PVY is managed in seed potatoes by visual inspection during growing season followed by post-harvest test. A national survey conducted in 2004-06 revealed that all strains of PVY were present but PVY^O was the predominant strain, which was further confirmed by a survey conducted in New Brunswick in 2009. The responses of 14 potato cultivars to five isolates belonging to four PVY strains varied significantly, ranging from local lesions to systemic necrosis and tuber symptoms depending on potato cultivar and virus strains. Field experiments on the current season PVY spread and the transmission risk by aphid species unveiled the presence of few potato colonizing aphids but many non-colonizing species, and among them, several were found to be PVY-positive by RT-PCR. Current season spread of PVY in the fields increased as the crop season progressed, and reached up to 40% at the time of harvest in some fields. PVY incidence determined from developing tubers exhibited a significant positive correlation with that from tubers after harvest using real-time RT-PCR. Application of mineral oil spray along with insecticide was found to be a very effective tool for the management of PVY in a growing crop.

Classification of PVY strains and new recombinants

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Currently, strains or strain groups of *Potato virus Y* (PVY) are classified based on two distinct approaches. The genetic classification groups PVY strains according to their ability to induce hypersensitive resistance (HR) responses in a set of potato indicators carrying three different *N* genes, hence five strains of

PVY are defined based on this system, PVY^O, PVY^C, PVY^Z, PVY^N, and PVY^E. The molecular classification groups PVY strains according to the sequence of their genomes which are often recombinant, built of segments of parental genomes, most often of PVY^O and PVY^N parents. The two most prominent recombinant strain groups are PVY^{N-Wi} and PVY^{NTN}, the latter associated with the potato tuber necrotic ringspot disease (PTNRD). Up until recently, the two classifications were not merged, due to unavailability of the PVY^Z and PVY^E sequences and lack of genetic typing of recombinant strain groups on potato indicators. In the past few years, both PVY^Z and PVY^E recombinant genomes were sequenced, and preliminary genetic typing of PVY^{NTN} was completed. This allowed development of a modified classification of PVY strains which combines genetic and molecular characteristics of recombinant and non-recombinant strain groups, and simplifies typing of new PVY isolates. Application of this modified classification to typing field isolates of PVY and search of the PTNRD genetic determinant will be discussed.

Potato seed certification and PVY

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New strains of *Potato virus Y* (PVY) are adding to the challenges faced by seed potato certification officials across North America. The use of the traditional visual inspection to detect these new PVY strains has not been effective. This lack of effectiveness occurs because the new strains, which often cause milder symptoms in common North American varieties than the original PVY strains, are simply more difficult or even impossible to see. Poor visual detection has resulted in the certification of seed lots with high levels of PVY and has been contributing to a general increase in PVY throughout the continent. The history of PVY in the Idaho Certification system will be discussed as well as some of the reasons the virus has been on the increase over the last two decades. In response to steadily increasing levels of PVY in Idaho seed, the industry recently abandoned the increasingly ineffective visual inspections for PVY in favor of the laboratory-based ELISA test for determination of PVY during the winter seed grow out. This change has been responsible for a dramatic decrease in PVY in the Idaho Seed System and could prove to be a template for the entire North American seed potato industry.

Unifying Concepts in Plant and Animal Vector Biology

A virus at the helm: Even plant-infecting viruses modify vector behavior!

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Pathogenic effects have been reported for vectors of both plant and animal pathogens. Modification of vector behavior that results from these pathogenic effects is of broad adaptive significance, because parasite fitness relies on passage to a new host which predominantly occurs during feeding. We have recently shown that vector infection with a plant virus results in altered feeding behavior. Infection of the vector *Frankliniella occidentalis* (Pergande) by the plant virus it transmits [*Tomato spotted wilt virus* (TSWV), Tospovirus: Bunyaviridae] results in dramatically altered feeding behavior that may increase vector competence. We found that infected males fed more than uninfected males, with frequency of all measured feeding behaviors increasing by up to 3 fold. Notably, infected males made almost 3 times the number of non-ingestion probes, the biting behavior that is most likely to leave plant cells intact and available for initiation of virus infection. Our results demonstrate that vector infection with plant viruses, as with animal-infecting viruses, can cause increased biting rates that may serve to enhance transmission. We hypothesize that this increase in probing behavior is a conserved trait among plant and animal-infecting members of the Bunyaviridae that has evolved as a mechanism to enhance virus transmission. Understanding the similarities and differences that exist between plant and animal virus vectors and building an awareness of the advances made in both of these fields will greatly benefit researchers.

LaCrosse virus modifies the behavior of its mosquito vector

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LaCrosse virus (LACV) infection modifies the mating behavior of *Aedes triseriatus* mosquitoes and conditions transovarial transmission efficiency of

the virus in nature. Mosquitoes infected orally with LACV are more efficiently mated than non-infected females. Infected females exhibited significantly greater insemination rates than non-infected females. Similarly, both field collected and laboratory-colonized transovarially-infected females mated more efficiently than uninfected females, conferring a fitness advantage for LACV-infected progeny. LACV is maintained in nature by stabilized infection in superinfected (SI+) mosquitoes, which efficiently transovarially transmit (TOT) LACV. Efficient TOT is conditioned by LACV perturbation of the *Aedes triseriatus* innate immune response in ovarian follicles, resulting in increased TOT-infected mosquitoes that mate more efficiently to maintain the SI+ phenotype in nature. The LACV - *Aedes triseriatus* relationship is truly remarkable.

What makes a vector a vector: The ecological and molecular basis of vector competence in planthoppers and thrips

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The interaction between arthropods and the viruses they transmit are complex, dynamic, and specific. Multiple biological, genetic, and environmental factors influence the competency and capacity of a vector to transmit a persistent-propagative virus. The first and most simple requisite is coexistence in time and space. The arthropod and virus must share the same plant host for some period of time for virus acquisition and transmission to occur. Once acquired, the virus must traverse barriers in the midguts and salivary glands and replicate to sufficient levels to assure transmission to a new plant. Within the vector, the virus must also survive without significantly compromising vector life-span and fecundity. Multitrophic interactions also influence vector capacity, and a virus-induced increase in plant host suitability may be one mechanism to overcome potential negative effects of virus infection on the vector. Specific examples and comparisons of vector transmission characteristics will be illustrated using a generalist virus-vector system (*Frankliniella occidentalis* and *Tomato spotted wilt virus*) and a specialist system (*Peregrinus maidis* and *Maize mosaic virus*). The application of molecular biology and -omics tools to these non-model systems has enabled

advances towards identification of viral and vector determinants of transmission, and these interactions will be discussed at the molecular and ecological scale.

The molecular basis of vector competence in mosquito-arbovirus interactions

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Although there are clear lessons to be learned from similarities in the molecular interactions between vectors and the viruses transmitted thereby, the potential research synergies in virus infections in insect-borne viruses of plants and animals are largely untapped. A distinct difference between vector-borne viruses is that the arthropod-borne viruses (arboviruses) of animals almost invariably depend on amplification in the vector for transmission, which is not the case for most vector-borne plant viruses. Even so, both plant and animal vector-borne viruses interact with, traverse, or infect and replicate in vector cells. It is increasingly clear that these interactions are not simple, benign events that go unnoticed by the vector. During infection with arboviruses including West Nile and Dengue (DENV), there is evidence for an active molecular arms race between virus replication and dissemination and the antiviral response in the mosquito; for example, DENV infection suppresses the Toll and Immunodeficiency (IMD) pathways, but the mosquito suppresses DENV, WNV and Sindbis virus infection with RNA interference (RNAi) and programmed cell death responses, particularly apoptosis. These innate immune responses are primary drivers for the compatibility of vector-arbovirus interactions and, therefore, of vector competence in blood-sucking arthropods.

Manipulation of host-derived olfactory cues by vector-borne pathogens of plants and insects

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An increasing number of studies indicate that vector borne pathogens can alter the phenotypes of their primary hosts in ways that influence the frequency and

nature of interactions between hosts and vectors. We have been exploring the effects of pathogens on host volatile emissions in a number of plant and animal disease systems. This work suggests that pathogens frequently alter host odors (as well as other aspects of host chemistry) in ways that influence the behavior of insect vectors and of non-vector insects. Moreover, pathogen transmission mechanisms appear to be an important factor shaping the evolution of effects on host-vector interactions. I will present recent results from work on viral and bacterial plant pathogens and from vertebrate disease systems, and discuss implications for disease transmission, ecology, and diagnosis.

Strategies employed by animal parasites to enhance vector transmission

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Parasitic protozoans and helminths that are transmitted from host to host by haematophagous insects have complex life cycles that often require a period of development (the pre-patent period), and sometimes asexual multiplication, in the host or vector before transmission can occur. For transmission to be successful the parasite must be in a suitable location and at a mature stage when the vector makes contact with the host. Biting insects are attracted to their hosts by the carbon dioxide, volatile chemicals and heat they emit and by their appearance. However, blood feeding is a risky foraging strategy and feeding decisions must be made to avoid host defence reactions. Using examples of parasites that infect humans causing tropical diseases such as malaria, leishmaniasis and filariasis, experimental evidence will be discussed that suggests that the attractiveness of infected hosts to the parasite's vector changes specifically when transmission stages are present. Likewise, evidence will be presented that shows that vectors infected with patent infections alter their feeding persistence and probing behaviour in ways that may enhance parasite transmission. Several parasites are also known to cause a reduction in vector fecundity and may alter the trade-off between reproduction and longevity, thus enhancing the prospects of transmission. The adaptive nature of these infection induced behavioural and physiological changes in host and vector will be discussed.

Disease Control and Pest Management

12th I. E. Melhus Graduate Student Symposium: Host Plant Resistance and Disease Management: Current Status and Future Outlook

Infection of blueberries by *Colletotrichum acutatum*: Host defenses, inheritance of resistance, and environmental effects

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A disease forecasting model for anthracnose fruit rot in blueberries was developed based on temperature, wetness duration, relative humidity, and wetness interruptions. The resistance response in the blueberry cultivar 'Elliott' was also investigated using suppression subtraction hybridization and several defense-related genes as well as abiotic stress-related genes were found to be upregulated in 'Elliott'. Some genes were related to oxidative stress and higher levels of hydrogen peroxide were found. Chemical fruit extractions were performed in 'Elliott' (resistant) and 'Jersey' (susceptible), and the methanolic extract was the most biologically active. Anthocyanins and flavonols were then quantified and identified in both cultivars using HPLC-MS. 'Elliott' fruit contained more anthocyanins than 'Jersey' fruit but the same compounds were found in both cultivars. However, two unique flavonols were present in 'Elliott'. A total of 26 blueberry cultivars were screened for anthracnose resistance using several different techniques. A cut-fruit technique was promising as a rapid screening method. Resistance was positively correlated with fruit sugar content but not fruit pH. The inheritance of resistance was also investigated by inoculating fruit from F-1 populations of specific crosses between resistant and susceptible cultivars; resistance ratings based on disease incidence were highly correlated with resistance ratings predicted from previous studies.

Proteomics-based study of host-fungus interaction between soybean and *Phakopsora pachyrhizi* using recombinant inbred line (RIL)-derived sister lines

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Phakopsora pachyrhizi, the causal agent of Asian soybean rust, has the potential to cause severe yield losses as all U.S. commercial soybean varieties are susceptible. In this study, ten soybean recombinant inbred line (RIL) derived sister lines of two populations (RN06-32-2 and RN06-16-1) were evaluated for differences in response to infection by *P. pachyrhizi* (Louisiana isolates). These lines, which had previously shown differential responses to Florida soybean rust isolates, were evaluated using Louisiana rust isolates under both detached leaf assay and greenhouse *in planta* inoculation conditions. Significant differences were observed among sister lines in their responses to *P. pachyrhizi* infection under both conditions. Lines 8-a and 8-b showed immune response; lines 15-b, 16-c, 94-b and 94-c showed resistant response; lines 15-c and 16-b showed moderately resistant response; lines 94-a and 8-c showed susceptible response. To understand the compatible and incompatible host-pathogen interactions at the molecular level, we conducted a time-course study of *P. pachyrhizi* infection and compared protein profiles of 8-a (immune) and 8-c (susceptible) lines using DIGE proteomics. Based on the gel analysis we identified approximately 70 differentially expressed spots between 8-a and 8-c lines with and without *P. pachyrhizi* inoculation and currently these protein spots are being sequenced using mass spectrometry.

Characterization of resistance of *Arachis hypogaea* to *Puccinia arachidis*

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In low-input peanut (*Arachis hypogaea*) producing countries, host resistance is the best option to manage peanut rust, caused by *Puccinia arachidis* Speg. We conducted field experiments to evaluate the resistance of CRSP breeding lines to rust, in 2010 and 2011 near Gainesville, FL and in 2011 in Tifton, GA. Disease severity was assessed using the 1-9 ICRISAT scale. In 2010, 18 of the 25 genotypes demonstrated resistance to rust, including genotypes highly resistance to rust late leaf spot. In 2011 the disease pressure in Gainesville was low and the disease epidemic started late in Tifton, leading to few differences in rust severity among genotypes. Peanut rust resistance is polygenic, thus we conducted detached leaf assays in 2011 to study the components of resistance to *P. arachidis*. Detached leaflets on moist sterile paper in Petri dishes, were inoculated, and incubated at 25 degrees. There seemed to be no correlation between the numbers of pustules developed and the pustule size, as genotypes developed both few and many pustules, both large and small. RAPD and SSR markers linked to rust resistance, will be used to screen selected peanut genotypes, to identify rust resistance genes in these genotypes. To study the molecular variability of *P. arachidis*, several isolates have been collected over different peanut growing seasons in different countries. Multi-locus Sequence Typing and phylogenetic analyses will be performed to define the population structure, in relation to the geographical regions.

Transcriptomic and genetic approaches to define tomato resistance to the bacterial pathogen *Ralstonia solanacearum*

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Bacterial wilt disease, caused by *Ralstonia solanacearum* (Rs), is considered the most destructive bacterial plant disease due to the pathogen's broad host range, aggressiveness and worldwide distribution. Tomato growers suffer complete crop losses during severe epidemics. Control of this disease relies primarily on breeding for resistance, which comes from the quantitatively resistant tomato line H7996. H7996 is resistant to most tropical Rs but not the emerging race 3 biovar 2 (R3bv2) subgroup of Rs. To define the pathways that tomato plants use to resist Rs infection, we sequenced the transcriptomes of tomato roots from susceptible and resistant lines (Bonny Best and H7996, respectively) after infection by tropical and R3bv2 strains of Rs. These transcriptomic experiments also explored how an Rs virulence effector PopS targets specific defense pathways. These transcriptomes broadly describe resistant and susceptible plant behavior during early infection to define the plant defense pathways triggered by an important and widely-distributed pathogen and an effector, PopS. Our investigations revealed physiological factors with differential importance for pathogen virulence on susceptible and resistant tomato lines. Specifically R3bv2 requires plant-derived sucrose to overcome H7996 resistance and for competitive fitness during H7996 xylem growth. Overall, these findings provide significant advances in our limited understanding of tomato resistance to bacterial wilt.

Emerging Tools and Regulations Impacting the Enhancement of Disease Resistance Using Biotechnology

Using TAL effector nucleases for targeted genetic modification

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TAL (transcription activator-like) effectors of *Xanthomonas oryzae* pv. *oryzae* (Xoo) contribute to pathogen virulence by transcriptionally activating specific rice disease susceptibility (S) genes. TAL effector nucleases (TALENs) are fusion proteins of TAL effectors and the DNA cleavage domain of FokI nuclease. TALENs are capable of inducing chromosomal DNA double-strand breaks (DBSs) at specific sites. DBS repair can lead to efficient introduction of sequence modifications or insertions through homology recombination or mutagenic insertions/deletions through nonhomologous end-joining at the site of the break, making TALEN technology a powerful genetic tool. We exploit TALEN technology to edit the specific S genes in rice to thwart the virulence strategy of *Xanthomonas oryzae* and, thereby, engineer heritable genome modifications for resistance to bacterial blight. Multiple designer TALENs are custom-engineered to precisely edit the effector binding elements within the promoters of two S genes targeted by three TAL effectors of essential virulence of Xoo. The resulting promoter modifications in transgenic plants result in loss of inducibility of S gene by the cognate TAL effectors and concomitantly loss of disease susceptibility (or gain of resistance) to pathogenic Xoo. The TALEN gene constructs can be genetically segregated out in some modified plants. The results demonstrate the feasibility of using TALENs for targeted editing of important genes for crop improvement and also raise the prospect of producing genetically modified plants without a trace of "foreign" DNA left in the genome.

Reintroduction of genetically engineered potatoes into the U.S. market

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The spread in the production of potatoes from small regions in the Andean highlands to almost 50 million acres worldwide coincided with, and enabled, in part, the unprecedented population growth and urbanization of the 18th and 19th century. However, issues in traditional plant breeding have hampered efforts to meet evolving needs for agricultural sustainability, and currently available varieties lack many of the agronomically important traits needed to control farm input costs. Shifting consumer preferences, from high calorie

diets to foods that are rich in phytonutrients, represent additional issues and have lead to a decline in the consumption of potato in the United States. The easiest and fastest way of adapting existing varieties to the needs of the 21st century is offered by genetic engineering. Currently available input traits provide resistance against some of the most important pathogens, pests, and herbicides, as well as black spot bruise. Additional quality traits enhance the antioxidant power of potato while lowering their toxin-forming potential. In many cases, it is possible to improve varieties by transforming them with native DNA only. Surveys indicate that consumers prefer such intragenic approaches over conventional transgenic methods that introduce foreign DNA into the food supply. Several genetically modified potato varieties are currently being evaluated for commercial production. With improvements in biotechnology quality management and identity preservation systems, reintroduction of the first genetically engineered varieties is expected to occur in the next few years.

Virus-mediated protection of maize from *Ustilago maydis*

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The corn smut fungus, *Ustilago maydis*, is a global pathogen responsible for extensive agricultural losses. Control of this disease using traditional breeding has met with limited success because natural resistance to *U. maydis* is organ-specific and is quantitatively inherited. Here we present a transgenic approach using *Totivir* antifungal protein KP4 expressed constitutively in transgenic maize. Transgenic maize plants expressed high levels of extracellular KP4 with no apparent negative impact on plant development and displayed robust resistance to *U. maydis* challenges to both the stem and ear tissues in the greenhouse. More broadly, these results suggest that a high level of organ independent broad-spectrum corn smut resistance might be afforded by transgenic coexpression of KP4 and other *Totivir* antifungal proteins KP1 and KP6.

Historical perspective of regulation and deregulation of biotech crops

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Over 25 years have passed since recombinant DNA techniques were successfully used to introduce foreign genes into plants. Biosafety Level 3 was required to prevent release to the environment, which was prohibited by the early Guidelines for Research with Recombinant DNA Molecules promulgated by NIH under direction of the highest levels of the U. S.

government. All Federal-funded research agencies were asked to comply with the NIH guidelines. This position resulted from intense scientific discourse ending in the compromise to assess and manage potential and conjectural risks by containment, but enabling research to continue. The USDA agreed, but pointed out that prohibiting release would preclude the power of biotechnology to agriculture. Useful discoveries in biotechnology in many areas were soon recognized as marketable products, resulting in an analysis of extant Federal Agency authorities for regulation of pharmaceutical, industrial and agricultural products. The 1986 Coordinated Framework for Regulation of Biotechnology clarified regulation of crop plants across three agencies, with approval of all needed prior to commercialization. Small scale field tests of plants was regulated by USDA-APHIS Plant Pest Quarantine and at first required permits from APHIS as well as EPA. Risk assessment and risk management discussions have continued, both nationally and internationally. Perspectives and results enabling deregulation will be presented.

Current processes involved in biotech crop deregulation

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Fungicides to Promote Plant Physiological Benefits in Crops

Influence of ethylene inhibitors on plant physiology, biomass, and yield

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Doubling average crop yields in the next 40 years to feed a growing world population will require simultaneous improvements in genetics and crop management, as well as alleviation of biological and environmental stresses. Because many of the physiological responses to stresses are modulated by the plant hormone ethylene, we are investigating technologies that alter the level of, or the sensitivity to, ethylene as means of protecting the corn crop from stress. We have examined the competitive ethylene inhibitor 1-MCP, which decreases plant sensitivity to ethylene, aminoethoxyvinylglycine (AVG), which decreases ethylene biosynthesis, and strobilurin-based fungicides which in addition to disease control cause a late-season leaf greening thought to be associated with ethylene biosynthesis. We have applied these compounds at different growth stages, under different environmental conditions, and with varied crop management in order to evaluate when, and how, the control of ethylene can be used to improve the productivity of corn. Collectively, our data suggests that altering ethylene level (or sensitivity) alone cannot guarantee higher yields, but rather ethylene control in combination with multiple management factors that impact productivity has the greatest opportunity to increase biomass production and grain yield.

Limitations of small plots for crop enhancement effects in corn

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In the previous symposium on this topic in 2009, the main focus of my presentation was on the effects of strobilurin fungicides on various physiological processes in treated plants. Today's presentation will concentrate on elucidation of yield benefits from strobilurin fungicides as influenced by plot size. Data over three years, from independent and Syngenta small-plot trials, have indicated a 2-3 Bu/A increase in yield in strobilurin-treated plots over untreated controls. These results were similar to those reported by University researchers as non-beneficial to growers. By contrast, data from commercial plots (≥ 20 acres) over the same period have indicated yield benefits of 10-15 Bu/A. To explore these discrepancies in the magnitude of yield benefits, Syngenta conducted trials in 26 locations in 2011 across the corn-belt with 2 hybrids per location. To eliminate border effects, these were large plot trials (5 acres per hybrid). The yield results from these 52 hybrid-by-location replications were consistent with earlier data from commercial-size un-replicated trials. Quadris at V5, Quilt Xcel at R1, or the combination of the two provided average yield benefits of 5.2, 11.2, and 14.2 Bu/A, respectively, over the untreated control. These data from 2011 suggest that the earlier results from commercial-size plots were a good indicator of yield benefits from the application of Quadris and/or Quilt Xcel at the

USDA/APHIS has been one of three primary federal agencies involved with the regulation of genetically engineered organisms since 1987. Its primary roles relate to permitting to allow field trials of genetically engineered (GE) organisms and making determinations of non-regulated status, which can lead to eventual commercialization of products. The process of obtaining non-regulated status typically requires conducting one or more years of field research under APHIS' oversight (a permit or notification) followed by submission of a Petition to the Agency documenting why the GE organism does not pose a plant pest risk. Typical data submissions include molecular and environmental characterizations that describe both the genotype (i.e., information about the inserted gene(s)) and phenotype of the organism. The phenotypic characterization typically includes a comparison with a closely related organism lacking the new gene(s) or with a representative range of related organisms as controls. Comparisons encompass a number of characteristics (e.g., growth habit, vigor, fecundity, outcrossing, reproduction, biotic and abiotic stress adaptations, cultivation practices, potential impacts on non-target organisms, etc.). Following a positive finding by the Agency, the organism may be moved and used in the environment without further APHIS oversight. Other U.S. government agencies involved in regulation of GE organisms include the FDA (assesses food safety) and the US EPA (deals with safe use of pesticides and pesticide labels).

recommended timings. By inference, yield data from small plot trials may be less accurate indicators of the benefits of strobilurin fungicides on commercial corn production in the relative absence of disease.

Disease management vs. insurance applications: What have we learned about fungicide use in corn?

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The increased use of foliar fungicides in United States corn production is the result of several coinciding factors. These factors include the increased demand and value for corn, a shift in crop production practices that favor disease development, and the labeling and promotion of the quinone-oxidoreductase inhibitor (QoI) fungicide group for use in field crops. QoI fungicides, commonly referred to as strobilurin fungicides, are marketed for management of biotic and abiotic stresses, and are promoted to increase yield even in the absence of disease. An analysis of 10 years of corn fungicide data indicates that when final foliar disease severity is greater than 5%, the average yield response from a fungicide application is 9.6 bu/A. In contrast, fungicide applications made in low disease pressure environments resulted in an average yield response of only 1.5 bu/A. This analysis reinforces recommendations to use fungicides in response to disease pressure or a disease threat for optimum efficacy and profitability.

Review of large-scale field trials in potatoes

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Vydate C-LV (oxamyl, a carbamate) is widely recognized for the control of nematodes and insects. In potato evidence exists for nematode control with a concomitant increase in tuber yield. A special Local Need 24(c) was granted to upstate potato growers for the in-furrow use of the product in 2008. Vydate has unique systemic characteristics, such that when applied to the soil it is absorbed and translocated upward, and when applied to above ground tissue it moves downward into the root system for plant protection. Little attention has been directed to any side benefits that may accrue when Vydate is applied at-planting for its effects on soilborne pathogens. Field studies conducted in 2008 and 2009 using Chieftain (widely adapted) and Andover (vulnerable to stress) addressed the effects that in-furrow (1X) and subsequent lower canopy spray of Vydate C-LV (2X) would have on overall tuber appearance and the occurrence of black dot (BD), black scurf (BS), silver scurf (SS), and common scab (CS). Enhanced yields were noted with the Vydate treatments for both varieties in 2008 and for Andover in 2009. Significant improvement in tuber appearance was noted in both varieties in both seasons with either 1X or 2X applications. BD occurrence on both tubers and stems was markedly reduced for both varieties in both seasons, and especially so for Andover. Similar trends were noted for reduced occurrence of tuberborne BS and SS, and less so for CS.

Grafting as an Alternative to Soil Fumigation for Disease Management in Vegetable Production

The pros and cons of cucurbit grafting in the United States

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From its research beginnings in the 1920's, cucurbit grafting in Asia has now become the predominantly practiced growing method; currently 95% of watermelon and oriental melon are grafted in Japan, Korea and Taiwan. As a result of the phase-out or reduction of soil fumigants, especially methyl bromide, grafting has only recently been considered as a practice for cucurbit growing in the United States. Grafting presents a promising option for soil-borne pathogen control with such diseases as Fusarium wilt, *Monosporascus* vine decline, *Phytophthora* blight and root knot nematode. However, grafting enhances tolerance to abiotic stress, increases water and nutrient use efficiency, extends harvest periods, and improves fruit yield and quality in certain cucurbits. These appealing advantages, as well as the grafting successes in Asia and Europe, make cucurbit grafting an important topic of study in the United States. The problems associated with grafting in the United States centers around economics, namely, the cost of producing the grafted transplant. These costs include added facility and supply costs and increased labor demands. Added facility and supply costs stem from the need for space to grow twice as many seedlings per grafted plant as well as provide a controlled environment for graft healing. Finally, grafting requires an increase in labor, not only in the skill required to produce grafted transplants, but the increased costs under our standards and the availability of such labor. In order for grafting to be successful in the United States these costs need to be taken seriously and research conducted to reduce these costs. Current research involves mechanization, as well as new and more efficient methods. Cucurbit grafting is a promising process; requiring study to create both understanding and innovation that will allow the United States to use this tool to enhance the opportunities afforded the grower.

IPM diversification: Advancing the science and practice of grafting tomatoes to manage soilborne pathogens

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Soilborne pathogens persistently limit optimum production in fruiting vegetable production systems. Preplant fumigation has been a core management tactic but the phase out of methyl bromide and increased regulatory requirements has spurred increased interest in alternative or complimentary disease management practices. Use of grafted plants has been a core method to manage numerous soilborne pathogens in many tomato production regions of the world. The Southeast USA tomato production region offered an opportunity to evaluate the efficacy of selected rootstocks (RS) to manage a diversity of soilborne pathogens. Multiple on-farm-research and research station experiments demonstrated the efficacy of grafting to manage bacterial wilt (*Ralstonia solanacearum*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*, race 1) and root knot nematode (*Meloidogyne incognita*, race 1). Serendipitously, several RS were discovered to offer near complete control of southern stem blight (*Sclerotium rolfsii*) and multiple experiments confirmed efficacy, a first report of commercially useful resistance to this important pathogen. Verticillium wilt (*Verticillium dahliae* race 2) severity was suppressed using selected RS; mechanisms need to be elucidated. Assessment of the diversity, dynamics and pathogenicity of pathogens and increased knowledge of host:parasite interactions undergirds RS selection and advances the science of grafting as a viable IPM diversification tactic. Participatory research and outreach programs advanced the practice of grafting in high tunnel, open field, organic and conventional tomato production systems.

Grafting on hybrid squash and bottle gourd rootstocks to manage Fusarium wilt of watermelon

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Fusarium wilt of watermelon is caused by *Fusarium oxysporum* f. sp. *niveum* (FON). FON is not pathogenic on other cucurbit species, although it can infect them. As part of our research on cucurbit rootstocks, we evaluated if FON was able to infect rootstocks, spread to the *Fusarium*-susceptible seedless

watermelon scion, and cause wilt after inoculation in greenhouse experiments. Grafted seedlings were inoculated by dipping roots into a microconidia suspension of FON race 1 or 2. Two to 3 weeks later, scion stems were cut above the first true leaf, disinfested and cultured on Komada's medium. Inoculated non-grafted Tri-X 313 and Tri-X 313 grafted onto *Citrullus lanatus* var. *citroides* Ojakkyo wilted, while the other inoculated rootstocks and healthy controls were symptomless. Recovery from scions grafted onto Ojakkyo (90% for race 1 and 63% for race 2) and from non-grafted Tri-X 313 (90 and 91%) was greater in inoculated than control treatments and greater than the other five rootstocks. Mean recovery of *F. oxysporum* from scions grafted onto *Lagenaria siceraria* Emphasis, Macis and WMXP3945 and *Cucurbita moschata* x *C. maxima* Shintosa Camel and Strong Tosa did not differ between inoculation treatments or among the five rootstocks. In a field infested with races 1 and 2, <2% of Tri-X 313 grafted onto bottle gourd and hybrid squash wilted compared with 53% of non-grafted Tri-X 313. Grafted plants yielded more fruit than non-grafted and self-grafted controls ($P<0.01$).

Grafting as a production system component for nematode management in Florida vegetables

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Trials in Florida evaluated grafting for control of root-knot nematode (*Meloidogyne incognita*) in tomato, cantaloupe, and pepper. Pepper rootstocks assessed included 'Charleston Hot', 'Carolina Wonder', 'Charleston Belle', 'Mississippi Nemaheart', and 'Carolina Cayenne', which were resistant to galling. The non-grafted and self-grafted 'Aristotle' scion, and several other pepper rootstocks were susceptible. In inoculated microplot trials conducted with tomatoes, juveniles (J_2) of *M. incognita* in soil were similar among all tomato rootstocks, but the number of J_2 in roots was higher in non-grafted 'FL-47' than in any grafted rootstocks. In cantaloupe, *Cucumis metuliferus* rootstock reduced galling while 'Tetsukabuto' did not reduce galling or J_2 in soil or roots. Grafted tomatoes and melons in a double-crop field trial showed that 'FL-47' tomato on 'Multifort' rootstock had the healthiest roots, but the number of *M. incognita* J_2 recovered from roots was similar for all rootstocks. Galling was highest in non-fumigated plots with non-grafted plants. For melon, *M. incognita* J_2 in soil did not differ, but J_2 in roots were higher in 'Tetsukabuto' than in *C. metuliferus*. *C. metuliferus* had less galling following all tomato rootstocks and in all fumigant treatments compared with the non-grafted 'Athena' and 'Tetsukabuto'. Grafting can provide some control of root-knot nematodes under subtropical conditions and can be combined with other soil treatments for more effective control.

Grafting eggplant to manage soilborne diseases: An international perspective

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Eggplant (*Solanum melongena*) is one of the most important vegetable crops in South Asia, widely produced on marginal soils in tropical climates by resource-limited smallholder farmers. Bacterial wilt, caused by *Ralstonia solanacearum*, and root knot, caused by *Meloidogyne* spp., are arguably the most important soilborne diseases of eggplant in this region. In Bangladesh, losses of 30-100% due to bacterial wilt in eggplant have been reported. Consumer preferences for eggplant fruit shape, size and color vary widely within the region, resulting in a least 100 locally or regionally preferred varieties in Bangladesh alone. Therefore disease-resistant varieties released from breeding programs are not always accepted by farmers and consumers. Several rootstocks have been identified that confer resistance to one or both of these diseases, including *S. melongena* EG203 (AVRDC), and wild *Solanum* species *S. torvum* (turkey berry) and *S. sisymbriifolium* (sticky nightshade). The local variety 'Chega' grafted onto *S. sisymbriifolium* rootstock survived 22-29 days longer, produced 250-280% more fruit and provided 3-4X higher income than non-grafted 'Chega' in bacterial wilt-infested fields in two locations in Bangladesh. The potential for, and challenges to, widespread adoption of grafting technology among smallholder farmers will be presented.

Issues and Opportunities in Regulatory Sciences at EPA

Reviewing biopesticides in the United States

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Biopesticides offer attractive and environmentally-benign components in IPM strategies for organic growers, and include biochemical and microbial active ingredients, and plant-incorporated protectants. They are distinguished from conventional chemical pesticides by their natural occurrence, target pest specificity, low volume use, lack of persistence in the environment and in general, low toxicity to humans. Before a pesticide is registered in the U.S., risk assessments are conducted to ensure that there are no unacceptable risks to human health and the environment when the pesticide is used in accordance with label instructions. The U.S. Environmental Protection Agency (US EPA) uses a tiered testing paradigm for evaluating hazards and exposures of pesticide uses. Information and data necessary to assess hazards are established in data requirements designed to address the primary disciplines of product characterization and manufacturing, human health, and environmental effects. Data requirements are grouped in a tiered testing framework and potential risks are determined first from estimates of hazard and exposure under “worst-case” scenarios (Tier I). Subsequent testing (Tiers II, III and IV) may be required to assess adverse effects under more realistic use or environmental conditions, especially when lower-tiered studies suggest potentially unacceptable risks. The US EPA has over 30 years of experience in preparing risk assessments to inform its registration decisions for biopesticides. The presentation will provide an overview of

microbial pesticides and the tiered risk assessment approach for biopesticide regulation.

Biotechnology for disease management: Regulatory considerations

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Biotechnology, or more specifically, recombinant DNA (rDNA) technology, has demonstrated real promise for managing plant diseases through genetic means. To date, however, few of these rDNA-derived products have realized commercial success. The use of viral coat protein or other viral sequences as modulators of RNA interference mechanisms have worked well in potato, papaya, yellow squash and European plum for viral resistance. Of these, only papaya and squash are currently in commercial use with plum to follow soon. Other disease resistant plants are in development, such as peanut expressing oxalate oxidase providing resistance to *Sclerotinia minor*, and inverted repeat transgenes using an RNAi-based approach to silence mycotoxin production by *Aspergillus* and *Fusarium* in maize. These types of products would be licensed under FIFRA as plant-incorporated protectants (PIPs), which are registered as pesticides by the US EPA. PIPs consist of the genetic material and their expression products as expressed *in planta* for the management of pests, including plant disease organisms. Insect resistant maize plants expressing the δ -endotoxin from *Bacillus thuringiensis* have demonstrated reductions in fumonisin content of seed as a consequence of reduced insect feeding damage and subsequent fungal colonization. This presentation will focus on the regulatory aspects of the development of biotech derived products for plant disease management.

The National Clean Plant Network: Ensuring Disease-Free, Vegetatively Propagated Fruit Tree Planting Stock

The National Clean Plant Network

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The National Clean Plant Network (NCPN) was established by the Farm Bill of 2008. Members provide high quality plant material free of targeted pathogens that cause economic loss to agriculture. NCPN contributes to the global competitiveness of U.S. specialty crop producers by generating desirable planting stock, increasing yields of healthy, high quality produce, and establishing rigorous standards for clean plant programs. The network is coordinated by USDA's Animal and Plant Health Inspection Service (APHIS), the Agricultural Research Service (ARS), and the National Institute of Food and Agriculture (NIFA). Partners include States, universities, non-profits, and industry. To support NCPN operations, 19 clean plant centers or programs located in 15 States received around \$5 million in NCPN funding in FY 2011 for pathogen diagnostics, therapy, and establishing foundation plantings. To date, five specialty crops are fully operational under the NCPN banner: fruit and nut trees; grapes; citrus; berries; and hops. Other specialty crops are also considering program entry. NCPN stakeholders build and operate network governing bodies, identify emerging clean plant issues, and strengthen linkages to allied activities such as nursery certification programs. NCPN has also organized working groups, including clean plant education and outreach to industry.

Plum pox virus case study: The eradication road is paved in gold

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The National Clean Plant Network (NCPN) plays a critical role in decreasing the negative impacts of viruses spreading via distribution of propagative material. In the world of temperate tree fruit, the high costs associated with virus introduction have been illustrated recently in the story of *Plum pox virus* (PPV), first detected in North America in 1999 in Pennsylvania. Although the route of introduction has never been proven, it is assumed to have entered as infected plant material. Intensive survey indicated that the virus was confined to a relatively small area, so an eradication plan was implemented. The

eradication goal was achieved – but only after ten years, \$53 million dollars, and the destruction of 1600 acres of stone fruit orchards. The mission of the NCPN is to produce and distribute virus-tested stock. Because industry is a vital part of the network, the system is constantly adapted to meet industry needs, making it less tempting to move untested stock that may harbor virus. Because the NCPN supports strong communication among virologists, USDA, state certification personnel, and growers, it is uniquely suited to uncover new viruses making their way into the nursery stream. The NCPN also draws together a group that could be quickly mobilized for virus surveillance activities, if needed. For each PPV introduction that is prevented by the work of the NCPN, the network is paid for ten times over.

New threats on the horizon for the fruit tree industry

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Disease management options based on the use of pathogen-tested, clean material are essential to limit the dissemination of viruses, phytoplasmas and viroids, and to mitigate their impact on fruit trees. USDA recently created the National Clean Plant Network (NCPN) to help protect specialty crops from the spread of economically harmful pathogens. Through NCPN, Clean Centers for fruit trees (NCPN-FT Centers) ensure the availability and maintenance of disease-tested, clean foundation stocks and propagation material through extensive testing and pathogen elimination therapy. Coordination of diagnostic guidelines and establishment of national standards are also important NCPN-FT activities. Amid the numerous successes and accomplishments of NCPN-FT, pathogens that are known or not in the U.S. continue to threaten the sustainability of the fruit tree industry, stressing the need for continued efforts to (i) explore novel approaches to accelerate the safe introduction and distribution of pathogen-tested, clean propagation material, (ii) better harmonize certification efforts across regional and national boundaries, (iii) set realistic certification standards that include current pathogen detection technologies, (iv) coordinate the implementation of science-based eradication programs, and (v) communicate more effectively on the benefits of pathogen-indexed, clean material.

Diagnosing and cleaning up viruses in imported fruit tree nursery stock

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New technology is rapidly expanding new opportunities to assist clean stock programs in the detection and elimination of viruses from vegetatively propagated perennial plants. Viruses cripple the economic sustainability of all segments of the temperate tree fruit industry, reducing or eliminating profit margins from the production and sales of trees and fruit. Additionally, domestic and international movement of nursery stock becomes complicated or restricted by efforts to minimize further dissemination of disease agents. Since peak profitability occurs soon after new cultivars are released, clean stock programs must balance the speed demanded by industry and the exceptional hygiene required of the final product. Critical translational research is needed so that benefits of new technologies can be fully captured by clean plant programs. To assist in the adaptation of new detection strategies, the etiology of severe disease can now be established with the assistance of high throughput sequencing methods. Advances in the understanding of the innate plant virus defense systems are informing the design of effective virus elimination strategies to produce pathogen-free trees. Outputs of these activities will support agencies charged with verifying the absence of exotic pathogens in transported material, and will benefit innovative and progressive fruit growers trying to compete in the global marketplace.

Quantifying the economic benefits of the National Clean Plant Network for the tree fruit industry in the Pacific Northwest

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This project is an attempt to quantify the economic value of plant certification programs, specifically the NCPN, to the tree fruit growers in the Pacific Northwest through a Net Present Value assessment of the industry. Production budget models were developed for apple, cherry, pear and peach production systems based upon published Extension budgets, tree fruit growers, USDA market data, and Washington State tree fruit industry reports. Net Present

Value estimations were calculated using AgProfit™ software. The NPV estimate took into account production system cultivars and orchard architecture. Five scenarios were developed: 1) The current situation, 2) 10 percent reduction in yield and 7 percent reduction in harvest costs, 3) 20 percent reduction in yield and 15 percent reduction in harvest costs, 4) 30 percent reduction in yield and 20 percent reduction in harvest costs, and 5) a shift in production systems and cultivars back to 2001 conditions. These scenarios were selected based upon feedback from industry stakeholder that suggested certified plant material increased orchard health thus without the NCPN, orchard yields would be lower. Additionally, it was suggested certified plant material fostered investment in new cultivars and orchard systems due to less risk from orchard diseases related losses. The results estimate the current NPV of grower returns in the PNW to be \$2.6 billion (assuming an 8% discount rate over twenty years). Under the assumptions in this study, without plant certification programs, reductions in grower returns range from \$828 million under scenario five to \$4.7 billion under scenario four.

The industry's perspective on the National Clean Plant Network

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The US tree fruit industry is a very specialized area of farming that competes in a global market. Fruit varieties are licensed, marketed and sold to consumers in every part of the world. The ability to have clean foundation material available across international borders is essential to maintain our competitive edge. Additionally, fruit growers produce in very high density, intensely productive systems. Our plant material must be clean in order to maintain these systems. We will explore the past, present and future benefits of the National Clean Plant System for the US tree fruit industry.

Diseases of Plants

Advances in Detection Technologies: Application in Plant Pathogen and Disease Detection

Next-generation diagnostics: Eliminating the excessive sequence processing associated with next-generation sequencing using EDNA

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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 have been applied to diagnostics. The massive volumes of data generated by individual deep sequencing runs increases the likelihood of finding pathogens present at low levels. However, sorting through the volumes of data, particularly if diagnosis is the primary concern, can be cumbersome. Significant portions of any metagenome are irrelevant for pathogen detection, and the assembly of complete genomes is not actually necessary for pathogen diagnostics. The initial objective of this work was to develop bioinformatic tools for streamlining sequence data analysis for diagnostic purposes. The bioinformatic process, termed E-probe Diagnostic Nucleic acids Analysis (EDNA) avoids assembly and GenBank BLAST steps while successfully finding nucleic acid signatures of microbes of interest. *In silico* simulations indicated that EDNA was both sensitive and specific in the detection of viruses, prokaryotic and eukaryotic organisms. Initial experiments using infected plant samples suggest that EDNA can detect the same range of organisms in real metagenomes, provided the pathogen titers are sufficient. EDNA also features a high degree of flexibility, allowing adjustment of both specificity and sensitivity levels to suit the needs of the user.

All plant virus chip: Shifting from proof to use

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The need to screen increasing numbers of plant viruses in a given sample has resulted in the development of highly parallel methods of detection and identification such as DNA microarray and next generation sequencing. Based on our results with a prototype universal plant virus microarray (UPVM) here we present the development and validation of a full UPVM to detect and identify all known plant viruses, viroids and satellites. A total of 9600 60-mer oligonucleotide probes were selected from 302,230 candidates representing every taxon. After confirming the capacity of selected probes to identify and discriminate viral species by *in silico* analysis, probes were synthesised without any linker or modifications and spotted on poly-L-lysine coated glass slides. The UPVM was validated with non-amplified amino-allyl labelled cDNA from nine healthy plants and more than 30 plants infected with characterized viruses. Validation is in progress with more samples from ATCC and 'unknown' samples. High-titre viruses could easily be detected and identified. For low-titre virus samples several strategies including increasing total RNA for labelling, depleting the host rRNAs and random-amplification are being tested. Comparison of hybridization profiles of healthy and infected plant samples identified a few non-specific reactions. Microarray data was also analyzed by hierarchical clustering and a predictive algorithm, T-Predict, to identify one or more viruses in a mixed infection.

The results of QBOL deposited in the Q-bank database to support plant health diagnostics

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The rate of introduction and establishment of damaging plant pests and diseases has increased steadily over the last century as a result of expanding globalisation of trade in plant material, climate change, EU expansion, and by a recognised decline in the resources supporting plant health activities.

Furthermore there is a constant decline in the number of taxonomic specialists in the different disciplines (mycology, bacteriology, etc.) capable of identifying plant pathogens, and funds to support this type of work are difficult to obtain. Also the number of other specialists in phytopathology and other fields, which are vital for sustaining sound public policy on phytosanitary issues, are diminishing. These problems affect all members of the EU and other nations. In this context QBOL (www.qbol.org), an EU project on DNA barcoding, started in 2009 to generate DNA barcoding data of quarantine organisms and their taxonomically relatives to support plant health diagnostics. The data are included in a database, called Q-bank (www.Q-bank.eu). Q-bank now consists of a dynamic open-access database of quarantine plant pests & diseases and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections. Within Q-bank curators from many countries with expertise on taxonomy, phytosanitary and collection issues for the different groups have been appointed and links with other databases have been made; this in order to provide Q-bank an international role in supporting plant health agencies. The results of the QBOL project will be presented as well as the Q-bank database.

CANARY: Serological detection sees a new dawn

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CANARY (Cellular Analysis and Notification of Antigen Risk and Yield) is a new serological method invented by scientists at the Massachusetts Institute of Technology. The technology uses engineered mouse B-cells that express membrane-bound, pathogen-specific antibodies and a calcium sensitive bioluminescent protein. Cross-linking of the antibodies by even minute amounts of the specific pathogen leads to elevation of intracellular calcium and the emission of light. The amplified light output is detected using an easy-to-use luminometer. The method is fast, specific (equivalent to other immunoassays) and sensitive (similar to PCR), and has been used for medical diagnostics and monitoring of bio-warfare agents. USDA APHIS and MIT developed plant-pathogen-specific-cell lines for detection of *Ralstonia solanacearum*, *Xylella fastidiosa* CVC strain and to the genus-level for *Potyvirus* and *Phytophthora*, and are developing cell lines for Citrus greening and *Citrus leprosis virus*. We employed simple procedures for testing bacterial pathogens with less than a minute detection and demonstrated analytical sensitivity of 3 CFU/test for *R. solanacearum*. For detection of *Phytophthora* and viruses, capture protocols are used in the sample preparation to enhance the CANARY performance. CANARY has the potential to improve the detection of plant pathogens in many economically and environmentally important plant species.

Pathogen signatures—Beyond nucleic acids & proteins

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Plant pathogen detection and diagnosis has characteristically involved morphology, isolation and the use of polymerase chain reaction (PCR) or a form of serological detection on strips or in microtiter plates. While these are the standard tools of our trade and widely proven, new technologies used in other disciplines are worth looking into for advancement or supplementation of the tools used to perform these activities. New technologies such as isothermal amplification and CANARY B-cell technology are beginning to show promise for diagnosticians and also require fewer reagents, have smaller footprints and do not require advanced-trained personnel. Additional technologies can also be applied to the detection of plant pathogens based on target properties such as volatiles or other chemical signatures. The potential use of detector dogs combined with rapid traditional diagnostics may be a realistic solution for plant inspection stations or ports of entry in the future. The integration of technologies used for bio-warfare agent detection or point of care diagnostics with more traditional technologies will be discussed. In addition, the integration of several different processes used in traditional diagnostics into single simplified systems used for detection or isolation will be briefly presented.

Isothermal amplification: So many names, are there differences?

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Polymerase chain reaction (PCR) has been the method of choice for the detection of microbial pathogens and in many pharmacogenomics applications. The assay is dependent on a thermal cycler to facilitate repeated cycles of denaturation, primer annealing and DNA synthesis to generate diagnostic DNA products using thermostable DNA polymerases. Unlike conventional-PCR, isothermal amplification (IA) does not need a thermal cycler to amplify nucleic acid targets and allows simultaneous strand separation, primer annealing and DNA synthesis at a constant temperature. Loop-mediated isothermal amplification, helicase-dependent DNA amplification, rolling circle amplification, nucleic acid sequence-based amplification, nicking enzyme amplification reaction and recombinase polymerase amplification represent IA methods currently in use. However, they differ in the way amplification is achieved, use of gene specific primers and choice of DNA polymerases. Some protocols include other nucleic acid modifying enzymes and ssDNA binding proteins to work in concert with DNA polymerases. Amplified products of IA may look similar to those of conventional PCR, recognized by dsDNA binding dyes and fluorescent probes, or represent massive amounts of DNA with loopy structures detectable without any additional reagents. Despite some problems in adopting the IA assays for routine detection, they have the potential to provide high throughput screening without expensive instrumentation.

Bioenergy Crops and Disease

Bioenergy crops and disease agents: Research and industry status

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Considerable resources are being directed toward developing plants with high and/or easily convertible levels of lignocellulose to serve the bioenergy industry. With this allocation of resources comes a responsibility to fully assess factors that can seriously limit this potential. Disease is one of these factors and we currently have the ability to analyze the influence of disease on these plants proactively (i.e. prior to full commercialization) rather than reactively. This talk will introduce the plant species under consideration as lignocellulosic biofuel crops. Their potential for yield improvement and reduced recalcitrance to biofuel production will be presented. Since the following talks in this session will detail the microbes that infect these species and their negative influence on yield or recalcitrance these topics will be only briefly summarized here. As an interesting contrast, a short overview of the use of microbes to enhance bioenergy crop yield and study recalcitrance will be presented. Supported, in part, by The BioEnergy Science Center, which is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science, and the Samuel Roberts Noble Foundation, Inc.

The impact of disease on biofuel production

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Plant disease can reduce both biomass productivity per unit land area (e.g. grain or lignocellulosic biomass yield per hectare) and the potential biofuel yield of that biomass substrate per unit dry matter (ethanol yield potential per bushel of corn). Plant disease may also contaminate biomass substrate with microbial metabolites (e.g. mycotoxins) that reduce the value of biofuel co-products from a higher value feed ingredient to a product valued only for its thermal energy content. Environmental impact (LCA) and production economics of biofuels are strongly influenced by these three factors that are mediated, at least in part, by plant disease. Biofuels are in the midst of an intense period of technology development, scientific investigation, and socio-political debate of costs and benefits. The definition of biofuel is expanding rapidly beyond yeast catalyzed ethanolic fermentation of sugars derived from the enzymatic hydrolysis of starch biomass. Both new substrates (e.g. lignocellulosic biomass), new processes (e.g. lignocellulosic pretreatment technologies), and new products (e.g. isobutanol) are expected to be in commercial use within a matter of months. The environmental merits of biofuels are the subject of an intense popular debate and numerous scientific and engineering studies. The use of life cycle analysis (LCA) to evaluate

environmental impacts is now common place in both drafting legislation and rulemaking by enforcement agencies (e.g. USEPA and CARB). In this context of rapid change we will use the 1st generation biofuel ethanol as an example of the potential for plant disease to impact economic and environmental analyses of biofuels.

Viruses of bioenergy crops

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Present and future energy challenges including crude oil volatility, energy dependence, sustainability, and environmental concerns are the motivators for renewed interest in non-fossil fuel sources of energy. An attractive approach is to produce alcohol from abundantly available biomass, using their hemicellulose and cellulose as substrates. Biomass crops are usually not consumed by humans and livestock and those having huge biomass yield are attractive feed-stocks for cellulosic ethanol production. The most prominent bioenergy crops are *Miscanthus* species (*Miscanthus x giganteus*), switchgrass (*Panicum virgatum*), elephant grass (*Pennisetum purpureum*), energycane (*Saccharum* hybrid), and energy sorghum (sweet *Sorghum* hybrid); while the most prominent biomass trees are *Poplar* species and willow (*Salix alba* 'Vitellina-Tristis'). *Miscanthus* is not native to America and different varieties of this crop are being imported from Asia. Some of these crops are being improved upon to increase their biomass yield and reduce their lignin content to increase the efficiency of the downstream ethanol production processes. Viruses pose great challenges to biomass production because of their potential to reduce biomass yield if they cause dwarfism or induce intensive mosaic or necrotic symptoms. Introduction of these bioenergy crops, especially the improved ones, into new environments might make them more vulnerable to new viral infection. There are also the concerns that these crops may be reservoirs of potent viruses of cultivated food crops. Identification of potential viruses of these crops is vital for the control and management of future potential viral threats as the crops are cultivated in commercial scale, and in multiple locations and environments. In this presentation, I will report research findings on potential viral threats of some of these bioenergy crops.

The potential of increased virus susceptibility in grasses modified for biofuel production

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Human experience with crop domestication and agricultural improvement suggests that development of new crop species can shift virus dynamics in a region. Will development of modified native perennial grasses as bioenergy feedstocks thus shift virus dynamics in the US Midwest? Wild populations of prairie grasses have inhabited central North America for millennia. But selection for biofuel-favored traits such as reduced lignin content and fast growth will change these species' phenotypes and potentially their disease susceptibility. Here we consider the influence of selection for biofuel-valued traits in *Panicum virgatum* (switchgrass) on its interactions with one widely-distributed group of aphid-vectored viruses (Luteoviridae: Barley and cereal yellow dwarf viruses). A common prairie element, switchgrass is now a favored candidate for bioenergy feedstock. We examined relationships between switchgrass growth rates and tissue digestibility in field-grown populations that ranged from near-wildtype to highly selected cultivars and compared these traits with measures of switchgrass attractiveness to aphid vectors and susceptibility to virus infection. We found that vector preference and population-level virus infection rates were not strongly influenced by

foliar digestibility but increased with biomass accumulation rates. To reduce risk of pathogen movement from fuel to food crops, we recommend screening of new bioenergy feedstocks for virus susceptibility.

Hunt for sources of rust resistance in the bioenergy crop, switchgrass (*Panicum virgatum* L.)

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Several fungal pathogens have been identified on ornamental and native strands of switchgrass (*Panicum virgatum* L.). Diseases of switchgrass have been largely neglected and pathogens could become the major limiting factors to biomass quality, and yield of switchgrass; especially when planted in monocultures. Leaf and stem rust caused by *Puccinia emaculata* is a major emerging disease in switchgrass research fields of Oklahoma. To identify genetically diverse source(s) of rust resistance, we evaluated half-sibling families from upland (Summer and Cave-in-Rock) and lowland (Alamo and Kanlow) switchgrass populations in both field growth chamber assays. Our results revealed a high degree of genetic variation within and among switchgrass populations. Alamo in general showed moderate resistance to *P. emaculata*, while Summer was highly susceptible. These results suggested a potential for improvement of rust resistance via the selection of the resistant individuals within the population. In addition, we have also initiated programs to identify and incorporate traits that confer nonhost rust resistance. Using forward-genetics screen in *Medicago truncatula*, we identified an *irg1* (inhibitor of rust germ tube differentiation) mutant that failed to promote pre-infection structure differentiation of *P. emaculata* due to complete loss of the abaxial epicuticular wax crystals and reduced surface hydrophobicity. An update on integrated approaches undertaken to mitigate rust disease and will be discussed.

Response of sorghum modified for bioenergy to grain and stalk fungal pathogens

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Sorghum is a versatile feedstock crop for cellulosic biofuels production. Reducing lignin content results in increased ethanol conversion efficiency. Two mutations in lignin biosynthesis were used to decrease lignin content and alter its composition. Near isogenic lines were developed with *brown midrib (bmr)-6* and *bmr12*, which are deficient in cinnamyl alcohol dehydrogenase and caffeic acid *O*-methyltransferase, respectively. Assessing *Fusarium* spp. infection of field-grown grain showed a significant reduction in *Gibberella fujikuroi* isolates in *bmr* grain, as compared with wild-type (WT); in particular, *Fusarium proliferatum* colonization was limited in *bmr12* grain. *Fusarium* sp. cf. *bullatum* colonization was absent in *bmr12*, while this genotype was common in *bmr6* and WT grain. Peduncles inoculated with *Fusarium thapsinum*, *Fusarium verticillioides* or *Alternaria alternata* had significantly smaller mean lesion lengths on *bmr6* or *bmr12* plants, relative to WT. Basal stalk inoculations with *Fusarium* stalk rot pathogens showed that *bmr* lines were as resistant as wild-type lines. Responses of *bmr* plants to foliar pests, including *Colletotrichum sublineoleum* and chewing insects, provided further evidence that reduced lignin lines were not more susceptible, and in some cases were more resistant, to common sorghum pathogens and other pests as compared to WT lines.

Schroth Faces of the Future—New Frontiers in Plant Bacteriology

Minerals influence interactions between the bacterium *Xylella fastidiosa* and its host plants

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Xylella fastidiosa (Xf) is a pathogen restricted to live inside the xylem of the plant host or mouth parts of insect vectors. Once inside the xylem the

bacterium forms biofilms believed to be responsible for disrupting water flow to the aerial parts of the plant. Evidence from different laboratories indicates that water deficit is not the only cause of disease development. We hypothesize that infection with Xf causes changes in the mineral status of the host plant that are used by the bacterium to accentuate its virulence. Infection of *Nicotiana tabacum* with Xf caused significant changes in the mineral and trace elements (viz. the 'ionome') of the plant, in particular increasing Ca and Mg, and reducing K and P. Changes in the ionome observed under greenhouse conditions in tobacco were confirmed in field samples from various plant hosts naturally infected with Xf, including grapes, blueberries and pecan. In

vitro studies showed that specific minerals (notably Ca) added to the media increased the ability of the bacterium to attach to surfaces, form biofilm and move. These traits were studied using microfluidic chambers, where also the process of biofilm formation is being mathematically modeled. Responses to metals are being investigated in a collection of Xf isolates from diverse host plant and geographic origins. Our studies are characterizing how minerals, main components of xylem fluid, shape diseases caused by Xf in diverse hosts.

Draft genomic sequence of rice pathogens and nonpathogens: Insights in biology, diversity, and diagnosis

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The declining cost of sequencing has made bacterial draft genomes a valuable tool for functional, comparative, and diagnostic information. We recently obtained draft genome sequence from a collection of poorly understood strains of rice-associated bacteria: multiple diverse strains of the pathogen *Xanthomonas oryzae*, including variants from Africa and the United States, strains of the rice bacterial pathogens *Burkholderia glumae* and *Pseudomonas fuscovaginae*, and two previously uncharacterized species of nonpathogenic *Xanthomonas* commonly associated with rice seeds and leaves. The weakly virulent US strains of *X. oryzae* were highly divergent from other studied *X. oryzae* strains, suggesting a long existence in the US. These US strains lack full or partial transcriptional like effectors (TALEs) common to other *Xanthomonas* pathovars. The lack of TALEs made them a useful tool for studying individual TALE roles in a panel of rice cultivars, a study which yielded several novel insights into TALE functional variability among hosts and pointed to possible sources of resistance. Intriguing finds from the other pathogen and nonpathogen draft genomes will also be reviewed, along with implications for future research. Finally, a rapid automated pipeline was developed to design sets of specific diagnostic primers by comparing draft and/or completed genomes; performance analysis and testing of the primers demonstrated the program to be a highly efficient means of diagnostic primer design. These studies demonstrate how draft genomes of non-model international isolates, isolates of low virulence, and plant-associated nonpathogens can improve diagnostic test development and reveal secrets of pathogen biology and diversity.

Effector proteins as probes to understand molecular mechanisms underlying plant-bacterial interactions and as markers for detecting bacterial diseases

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Thousand Cankers Disease: A Threat to Eastern Black Walnut Throughout Its Native Range and Beyond

The distribution and impact of thousand cankers disease in walnut species in the western United States

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Thousand cankers disease (TCD) was likely present in black walnut along the Wasatch Front Range in Utah and areas of the Columbia Gorge and Willamette Valley in Oregon as early as the late 1980's. TCD in black walnut is now distributed in the West as far south as New Mexico, as far north as Idaho and Washington, and as far east as Colorado. Disease severity has intensified in communities where it has been found and in many locations mature black walnuts have been eliminated, or nearly so, by TCD. The walnut twig beetle (WTB) and cankers caused by *Geosmithia morbida* also have been associated with a decline of southern and northern California walnuts in their native range. TCD has not yet significantly impacted the English walnut industry in California, although individual trees have been killed. The WTB and *G. morbida* have not been found in the native range of little walnut, although this species is susceptible to the fungus. Both the WTB and *G. morbida* are widespread in Arizona walnut throughout its range in the southwestern US, but only in association with damaged or senescing branches. The population structure of *G. morbida* in the West is complex. Haplotype

Bacterial pathogens have evolved a multitude of effectors that exert essential virulence functions in plant hosts during infection. Since effectors directly target components of host immune system, research on how effectors promote infection provides critical information not only on strategies taken by pathogens to infect plants, but also on plant defense mechanisms. Using both model and natural pathosystems with high impacts in agriculture, research in my laboratory aims to functionally characterize effectors secreted through the type III secretion system. In particular, our research has been focusing on the *Pseudomonas syringae* effector HopZ1, which belongs to the widely distributed YopJ family of type III effectors. We identified the direct targets of HopZ1 in the natural host soybean. Our data demonstrated that HopZ1 directly suppresses the isoflavonoid biosynthetic pathway, thereby promoting bacterial infection in soybean. In addition to our long-term goal, which is to apply knowledge obtained from basic research on effector functions to the development of sustainable and novel disease intervention strategies, we are also committed to develop shorter-term applications that can immediately benefit growers. One such application is to use effectors to develop novel serological detection methods for endemic bacterial diseases. I will discuss our recent progress on marker development for citrus bacterial diseases including citrus stubborn disease and Huanglongbing.

***Streptomyces* secondary metabolism and scab disease development: To what extent are these two processes linked?**

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Potato common scab is an important crop disease caused by the *Streptomyces*, which are Gram-positive, filamentous soil bacteria. The best characterized scab-causing streptomycete, *S. scabiei*, has a world-wide distribution and is the main causative agent of scab disease in North America. A key virulence determinant produced by *S. scabiei* is the phytotoxic secondary metabolite thaxtomin A, which targets cellulose biosynthesis in the plant host. The production of thaxtomin A is controlled by the pathway-specific regulator, TxtR, as well as five additional regulators that are conserved in other *Streptomyces* species. In addition, the recently available genome sequence of *S. scabiei* 87-22 has revealed the presence of additional secondary metabolite biosynthetic gene clusters (SMGCs) that may contribute to the pathogenicity of this organism. One such SMGC is predicted to synthesize molecules similar to the coronatine (COR) phytotoxin produced by *Pseudomonas syringae*, and recent functional analyses have confirmed a role for this SMGC in *Streptomyces*-plant interactions. Current research is focused on elucidating the mode of action of these virulence-associated COR-like metabolites and how production of these metabolites is regulated in *S. scabiei*. In addition, the role(s) of other *S. scabiei* SMGCs in plant pathogenicity is being addressed in order to provide a more complete picture of the virulence strategies used by this pathogen during host-pathogen interactions.

diversity in isolates collected thus far from Arizona walnut, the putative native host of the beetle and fungus, is different from isolates collected from black walnut and other walnut species in the West.

From discovery to regulation: A pathologist's perspective of thousand cankers disease in eastern United States

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Thousand Cankers Disease (TCD), is an insect (*Pityophthorus juglandis*)/fungal (*Geosmithia morbida*) complex that threatens black walnut (*Juglans nigra*). In early August, 2010, TCD was confirmed in Knox County in east Tennessee and this was the first report of TCD within the native range of black walnut. Since then, TCD has been found in five additional counties in east Tennessee. In 2011, the disease was reported in Virginia and in Pennsylvania. TCD may be hard to detect because the initial symptoms mimic those associated with drought stress. Since much of the eastern U.S. had suffered from drought in the last seven years, many hardwood species have displayed symptoms consistent with drought and this may have allowed TCD to stay hidden for several years in affected areas. To date, all reports of TCD in eastern outbreaks have been in urban areas or in trees along roadways. No trees in a forest environment have had a case of TCD confirmed. Since *P. juglandis* is considered a native insect by APHIS, the disease has not been regulated on a national basis. Regulation has been left to individual states and regulation has not been consistent. For example, in Tennessee, counties

surrounding counties with known outbreaks of TCD are regulated. However, when a county with TCD is next to a county in another state, the adjacent county is not regulated since it is outside of Tennessee's jurisdiction. This may make quarantines ineffective.

Evolution, diversity, and ecology of the genus *Geosmithia* with emphasis on *G. morbida*

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Bark beetles are associated with diverse fungal symbionts. One of these fungi, with poorly known biology belong to the genus *Geosmithia* (Ascomycota: Hypocreales). The current knowledge is based on samples from western Palearctic, USA and several other regions. 50 species of the genus (22 published, 11 formally described) were found until now. *Geosmithia* spp. are typically associated with phloeophagous bark beetles, bostrichids and weevils, and over of 90% from the screened insects species were found to be obligate or facultative vectors. A derived ecological strategy, found in three *Geosmithia* spp., represents association with ambrosia beetles, where they act as primary ambrosia fungi. Several species occur on alternative substrates or can live as endophytes of healthy trees. One species, *G. morbida*, was proven to be a pathogenic fungus causing tissue necroses on walnuts. *Geosmithia* community pattern and presence of primary ambrosia fungi suggest the long-term and stable association between *Geosmithia* and bark beetles. *G. morbida* belongs to clade of hardwood-associated species. Major enzymatic activities, utilization of C, N, P, S sources (BILOG microarrays), secondary metabolites and antimicrobial activities of *G. morbida* are presented. Physiological fingerprinting places *G. morbida* among strict specialists confirming its specificity to a single host and long term co-evolution.

Life history of the walnut twig beetle, *Pityophthorus juglandis*, and its association with *Geosmithia morbida*, causal agent of thousand cankers disease in the United States

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The walnut twig beetle (WTB) is a native phloeophagous (phloem-feeding) insect that has recently been associated with the newly described fungus, *Geosmithia morbida*. This insect-fungal complex is known as thousand cankers disease. In northern California male WTB colonize *Juglans* branches and stem material by tunneling into the phloem where they produce an

aggregation pheromone that attracts females and more males. The pioneer males are joined by two or more females and together they construct a largely transverse gallery system in the phloem. Both sexes carry the conidia for *G. morbida* and the pathogen appears to be introduced into host *Juglans* phloem during gallery construction. The pheromone has been used to demonstrate that WTB flies nearly year round with trap catches absent in December and very low in January. Peak flights occur in May/June and August/September; another major flight occurs in March/April when overwintering adults emerge. Overwintering larvae develop in spring and likely emerge in May. In the summer, most of the beetles fly between 1800 and 2000 h (crepuscular flight), but this diurnal pattern appears to shift as temperatures cool in the fall. The bulk of the WTB responding to pheromone-baited traps are females (0.59 to 0.83 relative to total number of beetles captured). An analysis of larval head capsule widths showed that WTB has three larval instars. The efficacy of the pheromone for detecting WTB was demonstrated over a range of population densities in eastern and western states.

Thousand cankers disease: A recently emerging disease of eastern black walnut in the eastern United States

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Eastern black walnut (EBW) is an important forest and landscape tree in the eastern U.S. Since the discovery of thousand cankers disease (TCD) in TN in 2010 and subsequently in VA and PA in 2011, the threat of this disease to the EBW resource has been of increasing concern. The rate of spread of TCD in the East is yet to be defined. Many EBW trees have been killed by this disease in the West, often within a few years. As environmental conditions in the West are quite different than in the East, it is not clear if the progression of this disease in the East will be the same as in the West. EBW is a mesic tree species, native to moist coves and alluvial soils. In contrast, many EBW trees are planted in the East on sites that often experience seasonal drought or other stresses that may affect TCD development. In 2011 in TN, I observed little or no new development of TCD external symptoms (wilt and branch dieback) on EBW trees in research plots, and increase in live crown ratings (recovery) on some trees was observed early in 2011. The rainfall for the growing season was 53% greater in 2011 than in 2010, when severe TCD symptoms occurred. It is possible that TCD on individual EBW trees in the East will not be continuously progressive, as was for chestnut blight, but be intermittently progressive, depending on the occurrence of environmental conditions favorable to disease development. Movement of infested EBW products is of special concern for TCD spread to new areas.

Ecology and Epidemiology

Exploring the Micropolis: Sampling, Identifying, and Analyzing the Diversity of Microbial Communities

Metagenomics for complex microbial communities

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Molecular techniques have revolutionized microbial ecology and constant advances in next-generation sequencing have dramatically improved our ability to explore both dominant and rare members of microbial communities at ever-greater depths. Until recently, shotgun metagenomics of complex communities was largely restricted to gene-centric analyses that highlighted important functional distinctions between communities, but not between individual populations within those communities. Current gigabase and even terabase scale projects are overcoming the obstacles to genome assembly from complex metagenome data and enabling new levels of functional exploration. I will review the sequencing technologies in use for metagenomics and describe their application to complex communities including soils and sediments.

From metagenomics to metabolomics: Communication in the rhizosphere

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Small molecules rule the rhizosphere. This has been clear since the first definition of the rhizosphere in 1904, but it emerged much later that

microorganisms are central to the dynamic conversation that creates the metabolic profile of the rhizosphere. Although plants initiate the conversation by pumping photosynthate from their roots, microorganisms rapidly respond by modifying plant chemistry and contributing metabolites of their own. Roles of certain microorganisms and metabolites have been elucidated, but a comprehensive portrait of the microbial membership, chemical composition, and dynamic responses of the rhizosphere has eluded us. Recent technical advances provide a new opportunity to tackle the complexity of the rhizosphere. Analysis of 16S rRNA gene sequences provides a census of both culturable and as-yet-uncultured bacteria in the rhizosphere community. Metagenomic sequence analysis offers insight into the genetic potential of the community, and functional metagenomics provides an activity-based approach to discovering new genes from uncultured organisms through functional expression in surrogate hosts. New methodology for mass spectrometry now promises a complete metabolomic portrait of the rhizosphere in real time. These methods used in concert have the potential to reveal new dimensions of the rhizosphere, particularly its variation over space and time and responses to microbial perturbation.

Metagenomics in fungal community ecology—Combining new and old technologies to maximize our understanding of complex fungal communities

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Emerging DNA sequencing technologies are making it quicker, cheaper, and easier to generate massive sequence data from environmental samples. This

deluge of information can quickly overwhelm our capacity to interpret results, understand how fungal communities are structured, and identify keystone species. In this talk I will provide a perspective based on my research in natural ecosystems and based on studies of fungi that are not well represented in sequence databases. I will discuss how the new, enhanced sequencing capacity ironically increases our reliance on reference databases and therefore necessitates more molecular systematic studies of fungi as well as widespread sequencing directly from fungal herbaria and culture collections. I will also discuss the importance of phylogenetically-focused sampling and how combinations of molecular and non-molecular approaches could be used to enhance our understanding of environmental sequences. I will argue that fungal systematics and ecology are inextricably linked and that both are necessary to adequately comprehend the ever-expanding flood of new sequences.

New frameworks in disease ecology that address the micropolis

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The revolution in sequencing technologies offers an exciting new perspective on full microbial communities - and on both microbial effects on plant health and microbial responses to climate. Components of plant health and plant productivity can be considered part of the extended phenotype or phenome of environmental microbial communities. Progress in plant genomics supports new levels of characterization of plant responses to microbial communities, which have potential for construction of a more nuanced understanding of the extended phenome in the context of epidemics. Two types of network models may prove useful for conceptualizing the roles of microbial communities in disease ecology. In one type, nodes of the network represent individual taxa. Approaches for modeling the interactions of these communities may be analogous to genetic networks and food webs. In another type, nodes of the network represent physical locations. This case includes epidemic network

models of the movement of pathogens, and may be extended to include the movement of communities of microbes. When such models treat the host as habitat for within-host modeling, they may also include the movement of forms of information associated with microbes, such as microbial and plant signaling molecules.

Welcome to the micropolis: How metagenomics can enhance plant pathology research

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Although plant pathologists and microbiologists have been investigating the impact of soil microbial communities for several decades, we still have an incomplete picture of how microbial diversity affects crop yield, disease severity, and ecosystem function. As new sequencing technologies have become cheaper and more accessible it provides an opportunity to investigate the interactions between soil microbial communities, plant hosts, and the soil environment at greater spatial and temporal scales. While the power and depth of data generated from soil ecosystems is unprecedented, using these new sequencing technologies in the absence of generally acknowledged standards may prove counterproductive. In this talk I will address these issues within the larger context of soilborne and root diseases in agricultural and forest ecosystems, and discuss some of the key aspects of meta ‘-omics’ analyses and how they can be used to provide a more comprehensive picture of the complex interactions of soilborne pathogens with their biotic and abiotic environments. Finally, I will present examples of how these new sequencing technologies can be integrated into traditional plant pathology research to investigate many of the unanswered questions associated with how soil microbial communities effect plant production and how we can harness the diversity of these communities for improved crop production and ecological function.

It's a Mixed Up World: Hybridization and Horizontal Gene Transfer in Plant Pathogens and Endophytes

***Verticillium longisporum*—A hybrid pathogen with an expanded host range**

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Hybridization plays a central role in plant evolution, but its overall importance in fungi is unknown. It has been suggested that new plant pathogens could arise by hybridization between formerly separated fungal species. Evolution of hybrid plant pathogens from non-pathogenic ancestors in the fungal-like protist *Phytophthora* has been demonstrated, but in fungi, the most important group of plant pathogens, there are few well-characterized examples of hybrids. We focused our attention on the diploid hybrid and plant pathogen *Verticillium longisporum*, the causal agent of the Verticillium wilt disease in crucifer crops. To address questions related to the evolutionary origin of *V. longisporum*, we used phylogenetic analyses of seven nuclear loci and a dataset of 203 isolates of *V. longisporum*, *V. dahliae* and related species. We confirmed that *V. longisporum* was diploid, and originated three different times, involving four different lineages and three different parental species. All hybrids shared a common parent, Species A1 that hybridized respectively with Species D1, *V. dahliae* lineage D2 and *V. dahliae* lineage D3, to give rise to three different lineages of *V. longisporum*. Species A1 and Species D1 are as yet unknown taxa. *Verticillium longisporum* likely originated recently, as each *V. longisporum* lineage was genetically homogenous, and comprised Species A1 alleles that were identical across lineages. The three different hybrid lineages may differ in virulence.

Emergence of *Phytophthora* pathogens by hybridization

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Interspecific hybridization is known to be a diversifying force in plants and is increasingly being recognized as an important evolutionary process in the emergence of pathogens. There are multiple instances of interspecific hybridization in the genus *Phytophthora* and they suggest that hybridization

between *Phytophthora* species can lead to the emergence of pathogens with novel host ranges. Interspecific hybridization has been hypothesized to be a consequence of the global movement of *Phytophthora* species. Has interspecific hybridization contributed to the evolution and diversification of *Phytophthora* pathogens? These questions will be discussed in the context of known hybrids, with a focus on *P. andina*.

The role of horizontal gene transfer in bacterial crop pathogen emergence

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Traditionally, *Pseudomonas syringae* has been viewed as a crop pathogen and consequently research has focused on strains isolated from crops. However, sampling precipitation, snow pack, leaf litter, and surface water has revealed an immense genetic diversity of *P. syringae* in these non-agricultural substrates. Interestingly, very close relatives of the tomato pathogen *P. syringae* pv. *tomato* (Pto) have been found among the *P. syringae* in these non-agricultural substrates. Some of these strains are as aggressive on tomato as Pto under laboratory conditions. Multilocus sequence typing and analysis of selected virulence genes show that these environmental Pto-like strains share identical alleles with Pto at some loci and that recombination between Pto-like strains and Pto has occurred during evolution of Pto. The implications of these findings for crop pathogen emergence and evolution will be discussed.

Hybrids everywhere: The role of hybridization in the evolution of *Neotyphodium* grass endophytes

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Interspecific somatic hybridization has played a prominent role in the evolution of seed-transmissible, mutualistic fungal endophytes collectively known as the epichloae. Current theory suggests that hybridization events may provide increased herbivore protection for the host plant through pyramiding of bioactive alkaloid genes. Additionally, these endophytes typically lack a

sexual cycle and thus, hybridization may alleviate the destabilizing effects of deleterious mutations that accumulate in the fungal genome. Here, we analyzed the genetic diversity of epichloid endophytes infecting populations of a single host grass species endemic to California, *Bromus laevipes*. In total, 58 isolates representing samples from 12 populations were analyzed and grouped based on intron sequences of translation-elongation factor 1- α (*tefA*) and beta-tubulin (*tubB*). We also grouped the isolates based on both morphological characters and alkaloid chemotypes to evaluate congruency between classification schemes. Preliminary data suggest that some of these hybrids were formed from different progenitor species while others share the same progenitor species, but may have involved distinct genotypes with differing alkaloid profiles. These findings suggest that the diversity of hybrid endophytes within single host species can reflect that observed more broadly within the grass subfamily Pooideae.

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

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

“Left of Boom!” Information: Form, Content, and Use in Epidemic Prediction

Information in multiscale epidemiological models

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Food, fiber, and energy demands over the next 50 years call for substantial increases in agricultural productivity while addressing crucial issues of environmental impact and resource sustainability. One approach to this challenge is to design agricultural landscapes that are resistant to the establishment and spread of plant disease epidemics. Epidemic processes occur over different spatial scales, ranging from infection of an individual plant cell to the dispersal of propagules within and between continents. In contrast, practical limitations usually restrict epidemiological studies to a much narrower spatial scale. Thus, there is a great need to better understand how epidemic processes translate across disparate spatial scales. We will discuss modeling approaches that make predictions across spatial scales that are relevant to the influence of landscape heterogeneity on disease spread. The focus will be on the degree to which processes may be scale-dependent versus scale-neutral, how the issue of scale influences the impact of landscape heterogeneity on prediction of epidemic spread, and the degree to which predictions of different models are congruent. One clear conclusion of the modeling efforts is that there is a crucial lack of biological data to adequately parameterize and validate currently available models.

Transportation grids as early indicators and warning—The use of census and travel data for prediction of disease incursions

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Detection of initial introductions of exotic pathogens and pests is challenging because they occur in very low incidence. The earlier the detection, the more likely the pathogen can be eliminated or the epidemic slowed, lessening impact over multiple years. Finding point introductions across a broad geographic landscape of mixed agricultural and residential areas requires substantial manpower and fiscal resources, and often goes undetected for prolonged periods until incidence exceeds the lower threshold of sampling sensitivity. The Census/Travel model utilizes probable pathways, parses regions into smaller areas (ex. zip code), and predicts the most likely locations for introduction. This geospatial method uses US census and international travel data combined with a pathosystem's epidemiological characteristics, i.e., latency, reproductive rate, etc. The model generates a risk index map that is linked to a survey optimizer that calculates the number of samples to be taken in each area based on risk, estimates manpower and fiscal requirements and ranks foreign countries by their relative risk. The model is independent of pathosystem and can be used to predict introductions of vectored and non-

vectored plant, human, and animal diseases/pests. Risk maps have been generated for several exotic plant and human/animal diseases: ex. malaria, dengue fever, yellow fever, chagas, and Chikungunya, and has validated known points of introduction for prior disease introductions.

Emergence of unified concepts of disease in textual surveillance data

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Technology has increased the amount of text based information available for use in biosurveillance. Large text-based repositories are evolving to include relational databases of test results, citizen observations, social networks, expert opinion and news media. Additionally, database interconnectivity is increasing rapidly. Human health surveillance has pioneered syndromic analysis of diagnostic test results, pharmaceutical purchase, absenteeism in schools and the workplace, and other case count-based analysis to inform situational awareness. With the development of the National Plant Diagnostic Network Repository, national and regional syndromic clinic analysis is now possible. Initially, local repositories consisted of open form text that was unstandardized. Analysis showed certain words are used more frequently than others. As technology and data streams improve, and media becomes near-real time, it is possible to recognize indicators and warning of potential or emerging outbreaks earlier. Additionally, public media analysis indicated the progress of an epidemic can be discerned based on what terms the communicators choose. Such an understanding of a communicator's choice of terms can be exploited to identify epidemic stages and severity. Biosurveillance programs have developed “taxonomies” of word choices to identify escalation of an event and tipping points as very early indicators and warning of an outbreak. Syndromic and media case examples are presented.

Advantages and challenges of using Internet media for disease detection and tracking

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The use of Internet media data for the early detection and situational awareness of infectious disease events has evolved into a globally recognized field. Internet media has the advantage of being timely -- available in any language from local to international sources. Published success stories include tracking events associated with mass gatherings, pandemic influenza, the aftermath of natural disasters, food shortages and plant pest and disease outbreaks. However, sifting through the vast array of information on the Internet, ranging from traditional text media to social media is challenging, e.g., detecting a significant signal among the vast noise of misinformation and non-specific reports. In addition, identifying anomalous activity without an established baseline of media activity for a given disease in a particular region is difficult. Recognizing false positive and false negative events is also problematic due to the lack of official reports or delays in diagnostic testing. As such, applying traditional epidemiological analytical approaches to Internet data is often not possible. Despite these obstacles, many recent

advances in the field, such as mapping of Internet data using visual analytic tools, forecast modeling and social networking analysis have demonstrated the great potential of media as a real-time complimentary approach to traditional public health methods of disease surveillance.

Putting information to use: Decisions at different scales

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Information pertaining to crop health may be gathered at the field, district, eco-regional, or global scales. These scales have been addressed in the case of rice in tropical Asia. This allowed cross-scale comparisons of crop health information and management. At the field scale, cropping practices (e.g., crop

rotation, crop management), i.e., medium to short term decisions have a strong bearing on crop health. At the district scale, results further highlight the links between production situations and crop health, and the emergence of new crop health problems linked with technology shifts (e.g., new plant material). At the eco-regional to global scales, simulation and GIS analyses suggest that eco-regions can be grouped according to epidemic risks (ER) and their attached uncertainty for five very different rice diseases. This latter scale brings about risk categories depending on diseases, i.e. (1) world areas of disease endemicity (high ER mean and low ER variance), (2) areas with low ER mean and low ER variance, and (3) areas where ER is variable, and ER variance is high. Durable control measures are required in the first category, but not in the second. The third category implies the highest uncertainty level, requiring better prediction to deploy tactical and short-term decisions timely. Combining different geographical scales enables different systems perspectives for disease management, and information use along the decision time-line.

Resolving the Species-Population Interface in Asexual Fungi: New Tools to Address an Old Problem

Asexuality across the kingdom Fungi and the taxonomic challenges of species delineation

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Recent changes to the International Code of Nomenclature for algae, fungi and plants have significant implications for practical plant pathology. Other than the abolishment of Latin and the implementation of name registration, asexual genera will be integrated with sexual genera of fungi, and a single name will be used to communicate about species. By employing DNA-based techniques, genera and species can be linked in the absence of all stages of their life cycle. To complicate matters, however, many genera are either poly- or paraphyletic, and many pathogens are in fact species complexes. Even though it is clear that the phenotype conveys limited information about true genealogical relationships, close to 80 % of all novelties described per year still lack DNA sequence data, which represents one of the biggest challenges facing our community. Although the internal transcribed spacer region has been selected as the universal fungal barcode, it only successfully identifies 73% of the taxa screened across kingdom Fungi to species level, suggesting that secondary barcodes will be needed to provide accurate identifications. This is especially true for species in many important plant pathogenic fungal genera such as *Botryosphaeria*, *Calonectria*, *Cercospora*, *Colletotrichum*, *Ilyonectria*, *Mycosphaerella*, *Phoma* and *Pseudocercospora*. Based on these findings, it is clear that even though many diseases are associated with species complexes, some of these species are in fact also complex in that they have a complicated ecology and life cycle.

Using comparative genomics for species resolution in *Alternaria*

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Systematic study in the genus *Alternaria* provides a unique opportunity to examine speciation processes across different fungal lifestyles and microbial ecology. The genus encompasses nearly 200 species, but most fall into two very distinct sister clades: Section Alternata, which represents wide-spread facultative pathogens, and Section Porri, which represents host-specific facultative saprobes. Twelve species from each Section have been sequenced using Illumina Next Generation Sequencing technology and functionally annotated based on gene prediction in one reference genome for each Section: *A. arborescens* for Section Alternata and *A. solani* for Section Porri. Whole genome comparisons revealed orthologs in high synteny within groups rather than between, supporting Section designation. Homology was much lower among host-specific species within Section Porri than among nonhost-specific species within Section Alternata, suggesting ancestral barriers to genetic exchange by host despite common sympatric associations. Gene ontology analysis also revealed differential enrichment in certain functional categories, suggesting that each Section encompasses some distinct metabolic pathways. Genomic polymorphisms revealed most species are clearly delimited by host within Section Porri, but indicated genetic exchange among clusters of species within Section Alternata, which may support their collapse into fewer taxonomic units.

Challenges and opportunities for species recognition in *Fusarium* provided by genomics

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In the last twenty years, there has been a complete overhaul of *Fusarium* systematics at the species level, as molecular phylogenetic approaches have facilitated the recognition of species boundaries using evolutionary criteria. While genealogical concordance principles have provided excellent guidance for recognizing species boundaries, in cases such as the *F. oxysporum* complex and *F. solani* species complex, non-concordance of gene genealogies due to possible unorthodox evolutionary patterns have been confounding factors. In addition, complete genome sequences of members of these groups and associated experimental results have led to hypotheses that large portions of their genomes have origins outside *Fusarium*, and support the potential for horizontal gene transfer. Can genealogical concordance principles be utilized to recognize species boundaries in a high background of interspecific gene exchange? Can signatures of speciation events be identified from complete genome sequences in such a background? In this talk, I will develop a framework for addressing these basic questions, and propose some approaches that may allow species recognition in groups that show dynamic genome evolutionary patterns.

Cladosporium: Current concepts, diversity, and taxonomy

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By dominance of aerobiota and on plants and in soil, members of the genus *Cladosporium* (anamorphic *Davidiella*) are collectively amongst the most common eukaryotic life forms. Although G.A. De Vries (1952) delimited 23 taxa and M.B. Ellis (1971, 1976) 43 taxa within *Cladosporium*, the most well known guides to fungi in air, food, seed, and soil, as well as guides to fungi from veterinary or clinical settings, have usually provided descriptions for 2-4 species, most commonly *C. cladosporioides*, *C. herbarum*, and *C. sphaerospermum*. Some contemporary, licensed technology for molecular diagnosis of molds also uses only these three species names. However, these three species are best regarded as species complexes, each encompassing the taxon *sensu stricto* plus several to numerous additional named species, and sometimes with multiple distinct, but as yet un-named, clades. Internal transcribed spacer (ITS) regions are insufficient for delineation of species in *Cladosporium*, although species complexes are reasonably identified by ITS and individual species by multi-gene topologies. Well over 700 species names have been published since 1816. Determination of correct names for given taxa, and segregation of *Cladosporium*-like taxa, are on-going but sometimes impeded by absence or condition of type material.

Comparative genomics and bioinformatic tools for studying evolution and speciation in fungi

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The patterns of genome-wide variation from population genomic studies can be used to identify the basis for adaptation, dispersal range, and degree of hybridization among strains. *Batrachochytrium dendrobatidis* is a diploid chytrid fungus and an amphibian pathogen. It is currently one of the most important emerging invasive disease affecting biodiversity of frogs worldwide. Using whole genome sequencing we can extract patterns of population genomic to test the hypothesis that the fungus is cryptically sexual and undergoing recombination, or that the diploid represents an asexual lineage that undergoes a parasexual or other chromosome copy number based changes to shuffle genetic content. Further comparisons among closely related

species were used to polarize changes in the genome focus on likely recent adaptations that are specific to the pathogen. Using the map of polymorphisms from genome sequencing and genotyping, comparative genomics for gene order conservation comparisons, and phylogenetic approaches, we can identify loci that adhere to and violate expectations for genome evolution and indicate targets of selection or related to rearrangements that have impacted compatibility between strains or species. Tools for bioinformatics and databases and software to enable these comparisons for enabling these studies and recently developed approaches will be demonstrated.

Right of the Boom: Deciding to Act, React, or Let Go in a Fluid Data Environment

Even when data are fluid a decision must be made

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Regulatory agencies respond to the introduction of quarantine diseases in a complex and often rapidly changing environment. Biology of pathogen-host relationships, technology adoption, disease management practices, risk perceptions, risk aversion strategies, resources, and economics all play a role when deciding on the response that occurs to the Right of Boom! In the early stage of an outbreak, information is typically fluid and frequently very limited, conditions are dynamic, and external influences (political, regulatory, scientific, and industry) can change depending on authorities, resources, and relationships with each other and to the situation in question. While much depends on the stage of an emergency in progress, Plant Protection and Quarantine has developed a generic framework for decision making to increase the likelihood that responses will be consistent and predictable. In the very early stages of an emergency, however, decisions can be influenced by perceptions or other factors rather than analytically sound principles. The goal here is to explore how information flows and the fundamental structure for a regulatory support framework that adapts deliberately and predictably to a fluid data environment.

Use of law enforcement indicators and warning to prevent and respond to a crime

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As part of the FBI's top priority in investigating terrorism, the FBI investigates suspected terrorist attacks or attempted attacks on US food or agriculture. These investigations involve potential indicators that develop from a joint partnership with other federal, state, and local partners, the industry, public health, and academia. Discussion will include potential targets, groups or individuals that represent this threat, why the food and agriculture sector would be targeted, where information could be researched, threat agents of concern, and the impact this threat would have if an agroterrorism attack were to occur.

The role of epidemiology research in shaping regulatory plant pathology

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Regulatory actions in plant pathology are designed to minimize risk to trade, food security and economic vitality. Understanding the epidemiological aspects of a pathosystems allows regulatory agencies to respond in a scientific manner after an event occurs (Right of Bomb). Additionally, the use of science-informed decisions provide increased accountability for the policy regulatory agencies as response strategies are determined, and greater likelihood that mitigation efforts will be successful. For example, after several decades of Left of Bomb activities investigating the potential impact of soybean rust in North America, soybean rust was discovered in the U.S. in late 2004. During the 2004-2005 off season, a series of organized responses allowed for a coordinated effort to quickly register fungicides under Section 18 and Section 3 guidelines so that the soybean industry had the appropriate tools in place to combat the disease in the 2005 season. At the same time, a national *ipm*PIPE website was developed that conveyed regularly updated distribution information from sentinel plots established for the most part by the land grant universities and funded by USDA Risk Management Agency. This critical epidemiological data addressing the spread of the disease was

credited by the US Government Accountability Office of saving the soybean industry up to \$299,000,000 in reduced fungicide applications in 2005 alone. Other pathosystems will be discussed as well.

From boom to busted: Trade concerns and disputes under the WTO's SPS Agreement

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Every year disease outbreaks, of varying intensity, occur throughout the world from both unpredictable occurrences and known underlying risks. Many risks are exacerbated by globalization and the increasing volume of international trade. Emergency measures taken to control a problem and subsequent regulations established to prevent future issues must effectively deal with the risks based on the best available science under uncertainty. Despite these efforts, conflicts among trading partners still arise. This paper analyzes all Specific Trade Concerns citing the Sanitary and Phytosanitary (SPS) Agreement of the World Trade Organization (WTO) from 1995 to 2011. There are 327 concerns raised of which 81 have a primary concern regarding plant health regulations. Analysis will show the common types of dispute, countries involved, most contested rule violation, and the impact of trade patterns on dispute likelihood. A framework is presented to aid in regulatory design that considers international commitments in light of the patterns of dispute.

Making and implementing program decisions in regulatory plant pathology

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Regulatory plant pathology (Rpp) strives to modify human behavior as it relates to plant health. It can assume many forms besides the obvious quarantine in the quest to minimize pest damage. Major goals are primarily to prevent or delay infection, or secondarily to minimize the spread and impact of pests as much as possible. Combinations of pure Rpp with other means of plant disease management are common. Rpp problems are usually urgent due to the dispersal potential of the pest, the brief window of economic opportunity and the limited useful life of the commodity. Such conditions preclude long, thoughtful decision-making, and call for speedy responses based on information in hand. Anticipating and preparing for potential incursions is fundamental, but adaptations and adjustments on the fly are frequently required. Phytopathologists naturally tend to presume that biological scientific criteria for decision-making are the most important or even the exclusive criteria, and may be dismayed to learn that economics, ecology, logistics, politics, public relations and legal matters also have a say in the decision process. Subordination of biological components to these other criteria should be expected under certain circumstances. Advocating for the biological science aspects in the combined arena is vital. Rpp practitioners would benefit from more direct interaction, interdisciplinary training and cooperation in these other criteria to optimize regulatory decisions and implementation.

A case-based analysis of information sources, sinks, and loops in regulatory plant pathology programs

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The collection, meaning, quality and use, of information are major themes in the presentations during the Left of Boom and Right of Boom symposia. Formal information theory has not been widely used in plant pathology, but nonetheless impressive progress has been made in understanding what needs

to be known to assess risks from new disease outbreaks and how to use knowledge to manage outbreaks that do occur. Notwithstanding this hard earned empirical advance in methodology it is instructive to apply simple elements of information theory to typical decision situations in regulatory plant pathology in search of further gains and to identify generic concepts. We review the concepts of information entropy, channel capacity and relative entropy in the context of networks of decision makers who are collectively

attempting to manage uncertain situations. To illustrate possible applications for these information theoretic concepts we use a re-analysis of past decision-making problems in which several symposium participants were involved. Some of the individual information theoretic statistics we discuss are relevant to issues raised in other papers in the symposia and we refer to these where possible.

Molecular/Cellular/Plant-Microbe Interactions

Genetics, Genomics, and Proteomics Approaches to Elucidate Arthropod-Vector Specificity

The effect of temperature on '*Candidatus Liberibacter solanacearum*' gene expression

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Zebra chip is a devastating disease of potato. The putative causal agent, '*Candidatus Liberibacter solanacearum*' (Lso), is a fastidious bacterium transmitted by the potato psyllid *Bactericera cockerelli* Sulc. Little is known about this complicated vector-pathogen relationship, but temperature has been shown to significantly affect the progression of ZC disease development. In this study, novel RNA sequencing (RNA-Seq) technologies were used to monitor infected and uninfected potato psyllid gene expression changes reared at different temperatures. The overall pattern observed for psyllid and Lso gene expression at two temperature extremes (18 and 30°C), compared to the moderate or 'baseline' temperate (24°C, where potato psyllids have historically performed optimally) was analyzed with respect to the presence and absence of Lso infection. The results indicate that temperature has a profound effect on psyllid physiology, and also to some extent (as is possible to determine) on Lso gene expression owing to some bacterial transcripts inadvertently carried over during RNA isolation. Preliminary comparisons among Lso infected psyllids at the three temperatures tested indicate that some of the misexpressed genes are involved in psyllid development and in Lso pathogenesis.

Host switching in the vector-borne plant pathogen *Xylella fastidiosa*

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Insect-borne plant pathogens often have complex life histories. One important question for many of these pathogens is how to regulate the expression of genes required for colonization of plant and insect hosts. Another essential question is how to prepare for the switch from one host to another. The plant and insect colonizer *Xylella fastidiosa*, a bacterium that requires insect vectors to disperse among immobile host plants, is an ideal model system to address such questions. In insects and plants *X. fastidiosa* occurs in distinct phenotypic states that are controlled by a density-dependent signal (cell-cell signaling) and environmental cues (host polysaccharides). Because *X. fastidiosa* traits that are involved in plant colonization are incompatible with insect colonization, and vice-versa, transitioning between hosts represents an abrupt and absolute environmental change. Experimental data with this system will be used to highlight broader questions in the field.

Functional transcriptomics of *Begomovirus*-whitefly transmission

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Emergent plant viruses in the genus *Begomovirus* are transmitted by the whitefly *Bemisia tabaci* sibling species group, resulting in crop damage, worldwide. To gain insights into the molecular and cellular basis of whitefly-mediated begomovirus specificity, and to identify whitefly effector molecules involved in transmission we employed two complementary approaches, proteomics and transcriptomics. The partial proteome was determined by multidimensional chromatography and tandem mass spectrometric LC-LC-MS/MS analysis of whitefly adults and extirpated guts. To aid in functional genomics analysis and the development of a search library for protein

identification we determined the transcriptome for adult whiteflies and the gut of the AZ-B biotype of *B. tabaci*. Twelve MudPIT runs yielded 597 unique proteins. Approximately 500 unique proteins were identified from whitefly adults, while 400 were identified in gut samples. Proteins were identified by searching translated, annotated ESTs from well-studied insects (GenBank) and the whitefly transcriptome (19% annotatable), and proteins in the Insecta database. The greatest numbers of hits were to pea aphid, fruit fly, and then to body louse. Real-time PCR amplification of selected whitefly genes revealed differential expression in time-course AAP studies comparing viruliferous (SLCV) with nonviruliferous whiteflies suggestive of putative effector functions. [Note: The JK Brown, L Brecki, J Crowe, D Gang, and C Soderlund teams contributed equally to this research].

Using proteomics and mass spectrometry to explore the dynamic virus-vector interface

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A majority of plant viruses and a large number of important animal viruses are transmitted by insect vectors. Nearly all insect-transmitted animal viruses are internalized and circulate in their insect vectors, while plant viruses are divided between those that are carried on the cuticle linings of mouthparts and foreguts and those that circulate in their vectors. Members of the Luteoviridae are economically important viruses in staple food crops and are retained in the phloem of host plants. Phloem-retention facilitates circulative transmission by aphid vectors. This presentation will highlight our efforts to develop and apply advanced proteomics technologies to enable us to explore the dynamic virus-vector interface. Several examples of proteomics data will be discussed to illustrate the power of these technologies to further our basic understanding of the molecular pathways involved in circulative transmission in plants and aphids and the excellent agreement of our data with previously published studies on the biology of circulative transmission. Finally, examples from our data will also be presented to show how proteomics technologies can enable us to develop novel strategies that disrupt virus movement within and between hosts.

Comparative functional genomics to elucidate psyllid-'*Ca. Liberibacter asiaticus*' and *solanacearum* interactions

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The Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama and the potato psyllid (PoP) *Bactericera cockerelli* Sulc. are vectors of one or several '*Candidatus Liberibacter*' species that infect citrus and solanaceous crops, respectively. ACP transmits '*Ca. L. asiaticus*', the causal agent of Huanglongbing, or greening disease of citrus. PoP transmits '*Ca. L. solanacearum*', the pathogen associated with zebra chip disease of potato and vein-greening disease of tomato. To elucidate genes involved in pathogen infection of the psyllid host and psyllid-mediated transmission, we have employed comparative transcriptome profiling using infected and uninfected ACP and PoP nymphal and adult instars. Comparative *in silico* expression analysis within and between both psyllid species revealed conserved as well as unique transcripts. A number of genes involved in psyllid nutritional and innate immune system pathways were differentially expressed in infected and '*Ca. Liberibacter*'-free psyllids, making them candidates for functional analyses. Also gene expression profiles varied between infected adult-nymph species pairs, suggesting different cues may operate at different developmental stages of '*Ca. Liberibacter*' spp. psyllid pathogenesis. Transcript profiling for ACP and PoP vector-'*Ca. Liberibacter*' complexes will aid in elucidating and functionally characterizing genes essential for '*Ca. Liberibacter*' infection and pathogenesis of the psyllid host, and for psyllid-mediated transmission.

Pathogen Effectors and Host Targets

Hotspots in viral siRNA accumulation in maize

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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 fungal effector AvrPiz-t suppresses host innate immunity by targeting the RING finger E3 ligases APIP6 and APIP10 in rice

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Although the function of effector proteins of plant bacteria and oomycete pathogens has been elucidated in the recent years, the information for plant fungal effectors is still lacking. Here we showed that the avirulence effector AvrPiz-t from the rice blast fungus *Magnaporthe oryzae* preferentially accumulates in the biotrophic interfacial complex (BIC), and is translocated into rice cells. Ectopic expression of *AvrPiz-t* in transgenic rice causes suppression of the flg22- and chitin-induced reactive oxygen species (ROS) generation and enhances susceptibility to *M. oryzae*, indicating that AvrPiz-t has virulence function to suppress the PAMP triggered immunity (PTI) in rice. Interaction analyses show that AvrPiz-t suppresses the E3 ligase activity of two rice RING E3 ligases, APIP6 and APIP10, and in return, the two E3 ligases ubiquitinate AvrPiz-t *in vitro*. We also found that AvrPiz-t promotes the degradation of APIP6 and APIP10 *in vivo*. Silencing of *APIP6* and *APIP10* in the non-*Piz-t* background leads to a significant reduction of flg22-induced ROS generation, suppression of defense-related gene expression and enhanced susceptibility against *M. oryzae*. Interestingly, silencing of *APIP10* in the *Piz-t* background causes strong cell death and a significant accumulation of the Piz-t protein. Taken together, our results demonstrate that AvrPiz-t suppresses the host innate immunity through manipulating the two positive PTI regulators APIP6 and APIP10 in the non-*Piz-t* plants. In contrast, APIP10 degradation by AvrPiz-t in the *Piz-t* background leads to the accumulation of Piz-t and triggers a battery of defense responses to prevent the rice blast infection.

Effector promoters play a major role in delivery of *Magnaporthe oryzae* effectors into living rice cells

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Magnaporthe oryzae secretes effectors to cause the globally important rice blast disease. Some effectors that accumulate preferentially in Biotrophic Interfacial Complexes (BICs) enter the host cytoplasm across host-derived membrane, but other effectors that are not BIC-localized remain within the membrane. To determine gene sequences controlling effector BIC-targeting

and host translocation, we analyzed transgenic *M. oryzae* expressing chimeric genes between PWL2 (BIC-localized and host translocated) and BAS4 (nonBIC-localized and host non-translocated). The in planta localization patterns for the mCherry fusion proteins were determined by confocal microscopy. We have found that Pwl2p, when expressed under control of the BAS4 upstream regulatory sequence (URS), showed impaired BIC localization, host translocation, and avirulence activity. On the contrary, Bas4p, when expressed under control of the PWL2 URS, gained host translocation. We have also determined that reciprocal swapping of 5' UTR between PWL2 and BAS4 genes did not alter respective protein localization specificity. Thus, effector promoters play the critical role in determining the preferential BIC-localization and delivery of effectors into host cells. Consistent to this, PWL2 is strongly upregulated in the BIC-associated hyphal cells. New quantitative assays for effector gene expression levels and protein delivery into host cells will also be presented.

Pseudomonas syringae type III effectors: Evolution, distribution, and host targets of a bacterial Monkey Wrench Gang

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Over 30 closed and draft *P. syringae* genome sequences are now publicly available with 57 Type III effector families confirmed and likely representing the near-complete super-repertoire for the species. The type III effectors have proven to be important tools for deconvoluting the intricate network of defense responses in diverse host plants, and the accumulated data on their activities have revealed the central role they play in suppression of the 2-layered plant defense response composed of PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). A core set of effectors is widely distributed among the sequenced strains and acts by disrupting vesicular trafficking critical to effective PTI, while other less well-conserved effectors inhibit various stages in PAMP perception through interference with signaling kinases. As host plants have evolved to recognize and trigger immunity in response to PTI-suppressing effectors, additional effectors have been deployed by the pathogen to disrupt ETI, contributing to an endless evolutionary cycle of detection and evasion. Insights into coordinated functional roles of individual effectors are revealed through analysis of effector family distribution among the major *P. syringae* clades and by disassembly and reassembly of the effector repertoire of *P. syringae* pv *tomato* DC3000. Distribution of effectors among sequenced genomes and documentation of their cellular locations, molecular functions, and involvement in diverse biological processes as captured using Gene Ontology Annotation can be found at the PPI Website (<http://www.pseudomonas-syringae.org/>).

Functional characterization of the conserved modular domains found in the oomycete RXLR effector superfamily

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The Oomycota consists of a diverse grouping of predominately plant and animal pathogens, evolutionary distinct from fungi yet parallel in the mechanism of infection. A number of plant and animal pathogenic oomycetes deploy small-secreted proteins, known commonly as effectors, to modulate intrinsic host functions and defense responses. The genus *Phytophthora*, a key collection in the Oomycota, consists of several infamous plant pathogens that adversely affect natural ecosystems and domestic agriculture. Genome sequencing of several *Phytophthora* species has in part led to the discovery of a large super-family of putative effector proteins. The hallmark of this super-family is a highly conserved N-terminal RXLR motif. The RXLR motif from several oomycete effectors have been shown to facilitate translocation into host cells in a pathogen independent manner via phosphatidylinositol-3-phosphate (PtdIns-3-P) receptor mediated endocytosis. Interestingly, several plant mutualistic and pathogenic fungi also deploy RXLR-like effectors that enter via a PtdIns-3-P receptor mediated endocytosis pathway. Here we present a detailed NMR study of the RXLR effector Avh5 interactions with the head group of PtdIns-3-P as well as several functional assays characterizing the respective C-terminal domains of *Phytophthora sojae* effectors Avr1b and Avh5 to further elucidate the mechanism of effector cell entry and intracellular function.

Cyst nematode effectors and their targets

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Plant-parasitic cyst nematodes secrete effector proteins through their stylets into host root cells. These proteins are crucial for the development of specialized feeding sites (syncytia) and for mediating susceptibility through the suppression of plant defenses and manipulation of a variety of host factors. Functional characterization of effectors revealed the mechanisms by which cyst nematodes modify cell wall structure, negate host defenses, alter auxin and polyamine signaling and change plant gene transcription. Of particular interest is an altered expression of microRNA396 as a potential consequence of effector activity. microRNA396 was determined to be a key developmental regulator of syncytium formation. This microRNA post-transcriptionally regulates the expression of the Growth Regulating Factor 1 (GRF1) and GRF3

transcription factor genes in the syncytium. The miR396-GRF1/GRF3 regulatory unit is involved in the initiation of the syncytium induction/formation phase and then the transition into the maintenance phase. Genome-wide gene expression profiling revealed major roles of this microRNA396 regulatory unit in controlling host plant gene expression, explaining about 50% of the gene expression changes described in the syncytium. Ongoing functional characterizations of nematode effectors promise to provide an understanding of how plant-parasitic nematodes drive normal root cells toward novel developmental pathways required for successful parasitism.

Professionalism/Outreach

Everything a Scientist Should Know About Politics, Funding, and Public Opinion

Policy 101: A not-so-boring look at how government works

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In an increasingly bipartisan political climate filled with repeated stalemates over the budget policy of the United States and other countries, it is reasonable to ponder whether scientists can have an impact on governmental policies or on science, education, and extension funding decisions. Whether one is “inside the beltway” or beyond the suburbs of Washington, it is hard to not become disillusioned with the political process. Despite the disenchantment, with a little knowledge and the right tools, it remains possible for ordinary citizens and scientists to affect change in policies and funding. This presentation will provide a brief refresher on how laws are made, with a focus on the steps that lead to appropriation of funds for scientific research. The inner workings of the United States federal government will be viewed from an insider’s perspective. Importantly, a constructive discussion about engagement in the political process and the tools needed for engagement will be presented.

International funding cooperation

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Within an increasingly global context for research collaboration, science funding agencies in the U.S. and their international counterparts are employing a variety of different mechanisms to allocate resources for such collaboration, to facilitate the establishment of new scientific partnerships, to engage the next generation of scientists in international research, and to leverage resources for greater impact. This presentation will provide an illustrative overview of approaches being used by science funding agencies to develop such cooperative programs. And, it will describe specific initiatives of the National Science Foundation that are intended to advance opportunities for international collaboration in science, engineering, and education. International funding cooperation.

Getting engaged in public policy work is easy

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Public policy engagement (PPE), within the realm of plant pathology, is a mechanism to define a framework of ideas and values that can be adopted by government to positively impact regulatory measures, funding priorities and laws that address specific issues or needs of members of The American Phytopathological Society (APS) and their stakeholders. Contrary to popular belief, PPE extends beyond lobbying and involves a diverse continuum of activities. Whether you are engaging media-social or otherwise, educating stakeholders, disseminating research, sharing resources, building networks, or supporting grassroots organizations you are working along a range of activities that can influence opinions and promote ideas that affect public policy. One’s level of involvement in public policy is a matter of choice and can be as simple as writing a letter or sending a “tweet” to a decision maker or as involved as becoming an APS-Public Policy Board intern or board member or speaking in Washington. Public policy activities can occur on a local, state or national scale and engaging decisions makers within each division is equally important. Whether one dives right in or eases in slowly, engaging in public policy activities provides one with the opportunity to raise the profile of issues that are important to plant pathologists, stakeholders and the community as a whole.

Scientists: Almost as prestigious as firefighters

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How do you view the public? Surveys indicate that most scientists view the public as homogeneous, undereducated, and misled – at least when it comes to science. How does the public view scientists? The answer may come as a surprise. This presentation will explore misconceptions of both scientists and the public, while addressing the influence of these views on science policy. The relationships between science, the public, and policymakers will be reviewed from modern-day, historical, and global perspectives. The role of scientist as advocate will be discussed.

Practice and Management of Microbial and Plant Germplasm Collections

The National Plant Germplasm System (NPGS) and GRIN-Global

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The National Plant Germplasm System (NPGS) is a cooperative effort by public and private organizations to preserve plant genetic diversity. Federal and State personnel at 20 sites are responsible for 547,000 unique accessions of a wide array of plant genetic resources representing 14,325 species and 2,355 genera. Included are the National Center for Genetic Resource Preservation, which conducts preservation research and provides back-up for all collections; 18 active sites responsible for seed- and clonally-propagated species; and the National Germplasm Resources Laboratory, responsible for the Germplasm Resource Information Network, a system for collection and dissemination of germplasm information. Examples of integrated plant-associated microbial research with genebank collections will be presented.

Collections of plant-associated microbes are very important; no collection is prepared to accept and maintain the variety of material being isolated and characterized by researchers. The NPGS, in partnership with the Global Crop Diversity Trust and Bioversity International, developed the GRIN-Global System to provide a database flexible, multi-lingual, license-free information management system to meet global plant genebank needs. Released internationally in 2011, NPGS staff members are working to implement GRIN-Global. This system should be assessed for its potential value for managing information associated with other biological collections, including microbes.

Management of germplasm collections and associated data via informatics tools: Opportunities and challenges

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Germplasm collections often connect past, present, and future research endeavors by preserving key research specimens and making them widely available. They also serve as a critical reference resource for taxonomic

research, preserve specimens from biodiversity surveys, and supply materials for various practical applications. To fully realize the value of germplasm collections, we should go beyond mere physical preservation. These collections must be connected intimately to active research programs and their associated expert personnel and data resources so as to ensure their continuous growth and integrity. Since many different types of data are generated using germplasm collections, resulting data should be organized in a format that is readily available and should also be linked to individual specimens. Given the need for maintaining and integrating diverse and large datasets to support research communities, it is essential to establish a cohesive and user-friendly cyberinfrastructure supporting the management of collections. Using a few projects as examples, I will present several key issues in creating value-added germplasm collections via the use of informatics tools.

Plant germplasm curation—Best practices

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The curator of any collection of living organisms, be they microbe, animal, insect or plant, has the ultimate responsibility for the maintenance of the genetic true-to-type that is inherent in each individual accession in their collection. With plants, field regenerations are required for larger collections, and challenges with plant size, reproductive system, pollination and maintenance of genetic integrity are dealt with using isolation tents, individually introduced pollinators, hand pollination and hand harvesting. Reproductive system and effective population size are also important considerations. Barcoding at all stages from seedbank to field to harvesting to cleaning to shipping aids in tracking accessions. These are not museum collections, consequently access to information is critical for users to decide which accessions to order, test and utilize, and of primary concern to derive maximum benefit from the collections. Transparent, web-based databases with easy-to-find, relevant accession characterizations, both phenotypic and molecular, greatly facilitate use. Finally, one cannot over-emphasize the crucial importance of backing-up collections in a geographically-distant secure site; if the active collection suffers a catastrophic loss, a complete set of accessions is available for current use and for future generations.

Experience with best practice guidelines for microbial germplasm repositories at the Fungal Genetics Stock Center

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The Organization for Economic Cooperation and Development published best practice guidelines for Biological Resource Centers in 2007. Similarly, the International Society for Environmental and Biological Repositories publishes best practice guidelines with regular updates. While the latter is more appropriate for collections of tissue specimens, and neither deal with herbaria or natural history collections, these together provide a virtual step-by-step guide for the management of ex situ microbial germplasm repositories. Because neither guideline gives detailed instructions on how to implement best practices it is valuable to share practices from existing collections. The Fungal Genetics Stock Center has over fifty years of experience with techniques and implements modern data and management practices. For example, the FGSC uses at least two formats to store every isolate, has an off-site back-up, works from established standard operating protocols, and has

clear strain accession and archiving policies. Moreover, the FGSC has trained staff and appropriate facilities to carry out the operations of a biological resource center. These simple examples form the foundation of best practice guidelines for culture collections.

From culture collection to genetic resource centre: The Dutch approach

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The CBS Culture Collection (1904), is the largest public service collection for living fungi in the world. CBS and other established fungal collections harbour a great hidden diversity that, within a relatively small time frame, can most effectively be surveyed by a DNA barcoding approach. CBS has therefore acquired funding to generate DNA barcodes of its entire collection. Faced with a great diversity of undescribed and cryptic taxa, taxonomic types have the prominent function of anchoring existing species names. Taxonomic type sequence information is rare, because few types have to date been subjected to DNA sequencing. The barcode sequences of the more than 8000 ex-type cultures will be of tremendous value for fungal taxonomy and its user community. Furthermore, the CBS yeast collection holds almost 9000 strains including the 2240 ex-type strains of all described species. The resulting barcode data set will be an invaluable reference source for yeast research. For more than 100 years, strains entering the CBS collections have been identified based on state-of-art techniques at the time of accession. DNA barcoding the holdings will allow to update identifications and to recognize strains in need of further research, which will not only increase the value of the collection, but will aid the user community. The DNA barcoding approach and online database identification tool will also prove invaluable to confirm the identity of strains selected for whole genome analysis.

DNA barcoding and next-generation sequencing—Opportunities and challenges for reference biological collections

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With the advent of DNA barcoding and next generation sequencing, reference biological collections have never been as relevant and important. In Canada, significant investments are currently being made to process large numbers of accessions for barcoding and to sequence the genomes of high risk plant pathogens. DNA barcoding can be seen as horizontal genomics whereby one or a few short markers are sequenced by Sanger technology for a very large number of accessions. In order to have the “barcode” keyword, sequences in GenBank must be from an approved marker, must be from a properly vouchered specimen or strain, and must have electropherograms attached. The International Barcode of Life (iBOL) initiative is supporting such activity and receives a very significant portion of its funding from Genome Canada. There are currently over 250,000 genuine barcodes in GenBank and 1.5M in the database of iBOL. Detection of microorganisms can now be done by matching millions of sequences generated by new technologies to reference barcodes. The cost of whole genome sequencing is also coming down very rapidly, making it possible to sequence the genomes of all species of a genus. Maintenance and support of reference biological collections are essential in this new era of barcode and genome sequencing as they provide the foundation to a new rapidly evolving technology. Without the reference collections, the science coming from next generation sequencing is difficult to interpret or even irreproducible.