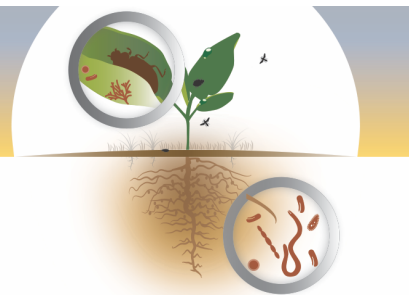


Phytobiomes 2015

Designing a New Paradigm for Crop Improvement

June 30 – July 2, 2015 | Washington, DC



Presentation Abstracts

Leaf endophytes modify *Melampsora* rust disease in *Populus*

POSY BUSBY ECA

Duke University, Durham, NC USA

Kabir Peay

Stanford University, Stanford, CA USA

George Newcombe

University of Idaho, Moscow, ID USA

Non-pathogenic microfungi known as endophytes are ubiquitous in plant leaves. In controlled, manipulative experiments endophytes can decrease or increase disease severity. However, whether leaf endophytes influence disease severity in agricultural or wild plant pathosystems is not well known. We used both culture-based approaches and next-generation DNA sequencing to characterize the fungal leaf microbiome of *Populus trichocarpa* in wild populations throughout the Pacific Northwest (USA) and to determine how common leaf endophytes influence the severity of a major leaf rust disease of *P. trichocarpa*, *Melampsora*. We observed both positive and negative correlations between the relative abundance of common endophytes and rust severity in wild trees. In both greenhouse and field inoculation experiments we confirmed that the endophytes modify rust disease severity in the predicted directions. Our results support disease modification by *Populus* endophytes both in the wild and in plantations.

Listing is organized alphabetically by presenting author's last name, which is noted in all caps. Abstracts are published as provided by author.

Posters will be available during all meals and breaks on first two days of the meeting in Congressional Hall AB. Authors will be present at their posters during the reception on Tuesday, June 30 from 6-7PM.

ECA = Early Career Awardee

The microbiome of cultivated rice: Structure, variation and assembly of the root-associated microbiota

JOSEPH EDWARDS ECA

University of California, Davis, CA USA

Root-associated microbes (i.e. the root microbiome) offer functional capacities to host plants not encoded within the hosts' genome. In particular, plants depend on interactions with root-associated microbes for nutrient availability, disease suppression, and growth promotion. The vast majority of root-associated microbes remain unidentified, and the organization and acquisition are poorly understood. We have conducted an extensive study of the root-associated microbiomes of cultivated rice by culture independent approaches. Using high-throughput sequencing, we have identified over 250,000 microbial taxa that are organized into three distinct compartments of rice roots: the rhizosphere (the soil adjacent to the root), the rhizoplane (the root surface), and the endosphere (the root interior). We analyzed the de novo assembly of root-associated microbiomes of rice grown in the greenhouse, finding that microbiome acquisition occurs rapidly in axenic plants with dynamic changes over time. This data supports a multistep model for microbiome assembly from the soil involving recruitment for certain microbes into the rhizosphere, gating at the rhizoplane, and selective entry into the endosphere. Characterization of the root-associated microbiomes of field grown rice shows that the major factors underlying variation in root microbial communities are geography, soil type, genotype and cultivation practice (organic vs. conventional).

Designing robust synthetic microbiota for increasing plant productivity

OMRI FINKEL ECA Isai Salas González, Jeff Dangl

University of North Carolina, Chapel Hill, NC USA

Plant roots grow within complex microbial communities, forming interactions with the root and with each other, ranging from pathogenesis to mutualism. These hold a vast genomic functional trait reservoir that may be harnessed for improving crop performance. Due to the complexity of natural microbial communities, and the prevalence of metabolic exchange in these communities, such single strains are nearly always ineffective when applied as probiotics to plants growing in heterologous, standing microbial communities. As designing and testing microbial consortia is exponentially more complex than testing single isolates for a given phenotype, there is a need for formulating and testing design principles that will assist in constructing such communities. We designed and are now testing microbial consortia, guided by the prediction that the level of metabolic complementarity within the plant microbiome is predictive of the level of mutually beneficial interactions, and thus, of microbiome productivity. The productivity of a beneficial plant microbiome, should, in turn, increase plant productivity. In order to test this hypothesis, a diverse library of 200 genome-sequenced bacterial strains isolated from *Arabidopsis thaliana* roots is being used. Bacterial consortia were constructed in a way that maximizes the ranges of metabolic complementarity, as defined below. An array of gnotobiotic *A. thaliana* were inoculated

with these consortia and plant growth and transcriptional profiles are being measured. Linking genome-derived predictions of community function to measurable phenotypes will help us infer design principles that will be applied first in controlled settings and ultimately in field settings.

Increasing functional photosynthetic efficiency (PFE) and inducible resistance to drought by application of endophytic fungi to the root phytobiome

GARY HARMAN, Molly Cadle-Davidson

Advanced Biological Marketing, Geneva, NY USA

Maximum plant photosynthetic efficiency has proven recalcitrant to improvement through conventional breeding systems, and is beginning to form an upper limit to efforts to improve plant performance and yield. Moreover, functional photosynthetic efficiency (FPE) is less than the maximum because of obvious and nonobvious stress factors. Photosynthesis and photosynthesis machinery are highly susceptible to damage by reactive oxygen species (ROS). ROS can be produced under conditions of high light intensity (a nonobvious stress factor) and even more so, by obvious stresses such as drought. Conversely, beneficial fungi (*Trichoderma* spp.) colonize roots and increase basal levels of photosynthesis (45%). Plants have redox cycling systems for the maintenance of optimal redox levels in plants. However, these cycling systems are usually insufficient to cope with the high levels of ROS that occur even under good field growing conditions, much less under high levels of stress such as drought. *Trichoderma* species induce coordinated upregulation of plant redox cycling enzymes that can maintain and improve plant productivity in the presence of both obvious and nonobvious stresses. This upregulation is greater under drought than in its absence, and does not occur in the absence of the fungi. The result appears to be a priming effect analogous to that observed with disease resistance. The capabilities of these strains create new opportunities to enhance plant productivity through plant genetics as well as through changes in agronomic practices. This improvement of FPE has applications in reduction of CO₂ as a greenhouse gas.

Learning to harness the phytobiome by growing our understanding of plant-microbe interactions

CHRISTINE HAWKES, Hannah Giaque, Elise Worchel, Moriah Sandy

University of Texas, Austin, Texas USA

Plant-associated microbes can mediate how plants perceive and respond to the environment, which is particularly relevant for coping with environmental stress. Yet much of our understanding of these effects is piecemeal. Here we argue that to harness the phytobiome as a practical tool will require (1) the development of a predictive, mechanistic framework for the outcome of plant-microbe interactions, (2) the ability to scale that predictive framework to multiple interacting microbes simultaneously occupying the plant host, and (3) a focus on underlying mechanisms, such as microbial metabolites, that can be used for tool development independent of the microbes per se. We provide an example with widespread, horizontally-transmitted, foliar fungal endophytes and their role in plant drought tolerance. The phytobiome represents an innovative plant management strategy with the potential for rapid, scalable benefits, but these will only be fully realized if we can gain a systems-level mechanistic understanding of their interactions with the plant.

The phytobiome influences the development of immunocompetency in *Arabidopsis*

James Kremer, BRIAN KVITKO, J.P. Jerome, Sheng Yang He

Michigan State University, DOE Plant Research Laboratory, East Lansing, MI USA

The relationships of plants with microbes are not always friendly. There are many microbes that exploit plants pathogenically with dramatic and devastating agricultural consequences. To protect themselves from pathogens, plants deploy an innate immune system based on a complex set of “non-self”-recognizing immune receptors coupled to carefully balanced defense signaling pathways. Currently, it is not known what role the endogenous phytobiome plays in the development and function of the plant immune system. Therefore, we sought to develop a soil-based system that allowed us to study the role played by the phytobiome in the development of immune competency. We have developed the “flowpot” system in which sterile *Arabidopsis thaliana* are grown in bottom-irrigated, mesh-covered potlets using a sterilized peat-based soil. This system minimizes the abiotic effects of plant growth in sterilized soil and is compatible with the field-standard battery of immune and infection assays. An RNAseq analysis of microbe-free *Arabidopsis* indicated that the expression of many key innate immune genes was significantly reduced when compared with colonized plants. We also observed that microbe-free *Arabidopsis* have significantly altered defense hormone ratios. Microbe-free *Arabidopsis* respond less robustly to immunity-inducing signals and producing weaker immune outputs. Microbe-free *Arabidopsis* are also significantly more susceptible to pathogenic bacteria. Preliminary results also suggest that additional factors beyond individual defense pathways contribute to the increased disease susceptibility in microbe-free plants. Our results indicate that the flowpot system will provide a useful soil-based platform to dissect the functional interactions between phytobiome dynamics and plant biology.

Identifying Mechanisms of Root Microbiome Composition Control

SARAH LEBEIS, Sarah Stuart Sloan

University of Tennessee, Knoxville, TN USA

Sur Herrera Paredes, Jeff Dangl

University of North Carolina, Chapel Hill, NC USA

Derek Lundberg

Max Planck Institute for Developmental Biology, Tübingen, Germany

Tijana Glavina del Rio, Susannah Tringe

DOE Joint Genome Institute, Walnut Creek, CA USA

Although land-plants grow surrounded by diverse microbial soil environments, only strikingly similar sets of microbial taxa appear to consistently colonize the endophytic compartment (EC) of roots, suggesting selective pressures exist. Here we describe our efforts to define the plant- and microbe-driven mechanisms that control the root microbiome. Specifically, a survey of the 16S rRNA genes found in the EC microbiome of wildtype *Arabidopsis thaliana* and mutants in defense phytohormone biosynthesis and/or downstream signaling revealed that salicylic acid (SA) plays a significant role in EC microbiome community composition. These findings were confirmed when wildtype and mutant plants were inoculated with a well-defined mixture of ~40 bacterial root isolates and half of the plants were treated with exogenous SA, allowing us to untangle direct SA effects from plant-dependent effects on bacterial communities. Finally, we also explore how a particular microbial family, Streptomycetaceae, which commonly colonizes *A. thaliana* EC communities, might shape subsequent colonization of other microbial taxa by the secondary metabolites they produce. Together, these studies have begun to reveal molecular mechanism used to establish and maintain plant microbiomes, which dramatically influence plant health.

Fungal volatile organic compounds: mostly overlooked novel effectors controlling fungal interaction with plants and rhizosphere competency

NINGXIAO LI  Vasileios Bitas, Nate McCartney, Jung-Eun Kim, James Tumlinson, Seogchan Kang

The Pennsylvania State University, University Park, PA USA

The rhizosphere encompasses the root surface and the soil layer surrounding it and offers rich nutrients. Accordingly, the rhizosphere attracts and harbors diverse groups of microorganisms. In order to colonize the rhizosphere, microorganisms have to manage certain plant defense responses and secrete antimicrobials to inhibit the growth and development of other competing microorganisms. Compared to the nature and modes of action of various proteins secreted by microorganisms to colonize the plant, relatively little is known about what metabolites rhizosphere inhabitants employ to facilitate their colonization of the rhizosphere. Because volatile organic compounds can travel far from the point of production through air, liquids, and soils, biogenic semio-VOCs can mediate both short- and long-distance organismal interactions. Accumulated evidence including our data suggests that certain fungal VOCs manipulate the plant physiology or other microorganisms. Based on their economic importance and rhizosphere competence, *Fusarium oxysporum* and *Verticillium dahliae* have been studied to help understand how their VOCs affect the plant physiology and fungal root colonization. Our data show that most *F. oxysporum* and *V. dahliae* strains significantly enhanced the growth of phylogenetically diverse plants via VOC production. VOC-treated *Arabidopsis thaliana* displayed enhanced resistance to *Pseudomonas syringae* and increased salt tolerance. Genetic and histochemical analyses indicate that fungal VOCs affect multiple phytohormone signaling pathways. Through targeted mutagenesis, we began identifying fungal VOCs that affect the plant physiology, fungal root colonization and pathogenicity. Our work on fungal VOCs as effectors will likely reveal a novel mechanism that soilborne fungi utilize to interact with plants.

Characterization of the grapevine endophytic phytobiome and its influence on Pierce's disease development

M. CAROLINE ROPER, Paul Ruegger, James Borneman, Philippe Rolshausen

University of California, Riverside, CA USA

Pierce's Disease (PD) of grapevine, caused by the bacterium *Xylella fastidiosa* (Xf), is a major threat to the grape industry. In vineyards that are heavily infected with PD, there are interesting examples of vines exhibiting either no symptoms or very mild PD symptoms (disease-escaped). All vines in a vineyard are clonal so the differences in tolerance to the disease are likely not attributed to the genetics of the plant. We hypothesize that the microorganisms inhabiting the xylem in these disease-escaped vines are inhibitory to Xf and subsequently reduce disease severity, due to their shared ecological niche. The goal of this project is to characterize the microbial communities residing in PD-infected vines and compare them to disease-escaped vines. We aimed to identify beneficial organisms antagonistic to Xf. We characterized the fungal and bacterial endophytic communities using an Illumina MiSeq platform targeting the ITS and 16S rRNA genes, respectively. Pseudomonadales was the most abundant bacterial taxonomic group, and Pleosporales and Sordariales were the most abundant fungal taxonomic groups. Some bacterial and fungal phylotypes correlated either positively or negatively with PD severity. Furthermore, we identified a subset of the endophytic microbes that possessed strong anti-Xf properties and suppressed PD symptom development in greenhouse bioassays. We envision harnessing these microbes to construct a beneficial synthetic phytobiome that can be deployed into grapevines during the nursery propagation process.

Analysis of the Maize Leaf Microbiome across 270 Diverse Maize Lines

JASON G. WALLACE  Shu-Yun Chen, Mei-Hsui Su, Jeremy D. Pardo, Karl G. Kremling

Cornell University, Ithaca, NY USA

Nicholas K. Lepak, Joshua S. Budka, Edward S. Buckler

USDA-ARS, Ithaca, NY USA

Recent years have seen large research efforts focused on improving crops and management practices to increase food production and achieve global food security. These efforts, however, have largely ignored the interactions between crop plants and microbes, with the exceptions usually involving either diseases (e.g., rusts and blights) or a few specific symbioses (especially of rhizobia and mycorrhiza). It is increasingly recognized that the overall microbial community plays an important role in the health of both plants and animals, and understanding the crop-associated microbiome may lead to advances in crop management and production to better achieve food security in the coming decades. To help identify how host plant genetics interact with and shape a crop's microbiome, we collected leaf samples from across ~270 diverse maize inbred lines taken from the US maize germplasm collection. All samples were collected from the same field in August 2015 and analyzed by targeted 16S amplification and deep sequencing to identify the bacterial components of the leaf microbiome. By linking these data with genotype data from each inbred line, we are able to estimate the heritability of the microbiome (both overall and of individual components) and perform genome-wide association to identify host genetic influences on the microbiome makeup. Our results represent the most diverse maize microbiome analysis to date, and they help illuminate the interaction between maize plants and their leaf microbial communities. Additionally, they will inform future studies regarding how we can best manipulate the leaf microbiome to improve crop performance.

Understanding beneficial and pathogenic microorganisms that can impact production of forage crops

CAROLYN YOUNG

The Samuel Roberts Noble Foundation, Ardmore, OK USA

Year round grazing for beef cattle provides advantages to farmers and ranchers by limiting the amount and cost of supplemental feeding. Forage deficits often exist in the fall and late winter, and environmental conditions, such as drought, can reduce stand longevity. The Forage365 initiative developed for the southern Great Plains (Oklahoma and Texas) is a systems-based approach to enhance forage production by integrating perennial forages that will complement the warm season grasses and winter annuals currently in use. The interaction of microorganisms, such as symbiotic and pathogenic fungi and bacteria associated with these forage species, are also being explored. Beneficial fungal symbionts (*Sebacina* and *Epichloë* species) and nitrogen-fixing bacteria are being evaluated to lower agricultural inputs, such as fertilizer, and to provide protection against some biotic and abiotic stresses. To improve alfalfa production we need to better understand the interaction between this host and the fungal root pathogen *Phymatotrichopsis omnivora* that is endemic to the southern Great Plains. Aerial imagery is used to identify areas of stand loss, follow the pathogen progression within a growing season and determine the cost of lost alfalfa production caused by *P. omnivora*. Each of these plant-microbe interactions is being studied at multiple levels to gain insight into host performance under various environmental conditions.

Thank You Sponsors

This meeting was made possible in part by support from these important sponsors:



Poster Abstracts

Poster #100

Effect of Chelating Agents, Fungi and Native Plants in Remediation of Metals Contaminated Soils

SHAZIA AKHTAR, Shazia Iram

Fatima Jinnah Women University, Rawalpindi, Punjab Pakistan

In present study four peri-urban agricultural areas (Multan, Kasur, Lahore and Gujranwala) in Pakistan were selected and surveyed in April 2012. Total 138 soil samples, 131 plants and 52 wastewater samples were collected. Soil and waste water samples were analyzed for their physiochemical parameters and processed for fungal isolation. Plants samples were tested for heavy metals contents. In overall assessment Pb, Cu, Cr and Cd was showing high level of contamination in the studied areas. Maximum fungal diversity was found in Multan followed by Kasur, Lahore and Gujranwala. In second phase soil shacking and incubation experiments were conducted to evaluate the changes in Cu, Cd, Cr and Pb solubility by addition of different concentration of Ethylene dinitrilo tetra acetic acid, Diethylene triamine penta acetic acid, Nitrilo tri acetic acid and fungal spore suspensions of six metals tolerant species. It was found that with increasing chelating agent doses metals availability was increased. Shaking hours of 120 and 20-30 incubation days were noticed the best optimum value for further experiments. *Aspergillus* species were proved best for metals solubilisation from soil matrix. In third phase in-vitro experiments were conducted growing different seed varieties of local crops e.g wheat, maize, barely, bajra, sunflower, soybean and mustard. Root and shoot biomass was recorded and heavy metal concentrations were checked plants tissues. Higher biomass production was noticed in maize and mustards crops and these plants were proved good phytoextractants for heavy metals.

Poster #101

siRNA sequencing of RNA from infected Sweet potato leaves reveal symptomless Mastreviruses in an SPVD-like syndrome in Barbados.

ANGELA ALLEYNE, Cara Cummins, Michael James

The University of the West Indies, Bridgetown, Barbados

Sweet potato viruses are a major biotic constraint to global production of sweet potato (*Ipomoea batatas* (L.) Lam). In 2000-2003, a decline in yields by more than 50% was observed in some sweet potato fields in Barbados and symptoms of yellowing, swollen leaf veins and deep grooves on the tubers suggested the possibility of Sweet potato virus disease (SPVD). The Sweet Potato Feathery Mottle Virus (SPFMV) which has been associated with the Sweet Potato Virus Disease (SPVD) was suspected as the possible agent. SPVD however is a complex synergy of several viruses, in addition to SPFMV which is vectored by aphids. This study sought to characterize the other viruses in the SPVD complex affecting sweet potato in Barbados and confirm the presence of SPFMV in the SPVD-like disease by using PCR amplification of viral RNA and genomic siRNA sequencing of affected sweet potato leaves. Initial PCR of viral RNA using coat protein specific primers for SPFMV, SPV2, SPVG and SPCSV only detected the Potyvirus SPFMV. However, further genome wide sequencing of siRNA derived from total RNA extracted from the infected leaf tissue identified Mastrevirus genomes in all samples, and Pakyvirus and Ampelovirus as possible viruses in the SPVD-like complex in Barbados.

Poster #102

The Underappreciated Mycorrhizosphere

JEAN-MICHEL ANE, Kevin Garcia, Matthew B. Crook

University of Wisconsin, Madison, WI USA

The vast majority of land plants interact with mycorrhizal fungi and it is suspected that this symbiosis enabled the initial colonization of terrestrial environments. To date most microbiome studies of plant roots have ignored the role of mycorrhizal fungi in defining the rhizosphere community. Of those few studies that have considered mycorrhizal fungi, all have focused on culturable taxa or have relied on techniques with limited resolution (e.g. RFLP). Despite their limitations, these studies have identified a subset of soil bacteria, known as mycorrhiza helper bacteria (MHB), that interact with mycorrhizae and enhance their symbiosis with a host plant. Mechanisms that have been described so far include improving fungal germination and growth rate, increasing plant root cell permeability, promoting plant root branching, improving nutrient acquisition, and inhibiting pathogenic fungi (Artursson *et al.* 2006, *New Phytologist*). Bacterial lineages that have been found associated with mycorrhizae mainly belong Proteobacteria, Firmicutes, and Actinomycetes. Early in the evolution of fungi, these MHB likely gave rise to the endosymbiotic bacteria (Proteobacteria and Firmicutes) found in many AMF lineages and may even have been responsible for the associations between rhizobia (Proteobacteria) and legumes and between *Frankia* (Actinomycetes) and actinorhizal plants that eventually led to efficient nitrogen-fixing associations. In order to better understand mycorrhizal microbiomes, their ecological and evolutionary significance, and how they differ from plant microbiomes, we barcode and sequence the root microbiome of various host plants mycorrhized by ectomycorrhizal fungi, by arbuscular mycorrhizal fungi, or not mycorrhized, grown in the same soil.

Poster #103

Genetic diversity of *Fusarium* isolates in Pakistan

Rahat Ghaffar, WAHEED ANWAR, Muhammad Shafiq, Khadija Imtiaz, Muhammad Saleem Haider

Institute of Agricultural Sciences, University of the Punjab, Lahore, Punjab Pakistan

Fusarium is diverse Genera and its different species cause wilt disease in different trees, ornamental and field crops. In this study, different species of *Fusarium* isolated from Pakistan were obtained. For their molecular characterization, their internal transcribed spacer region (ITS) was amplified and their homology was determined with other *Fusarium* species of the world. It was observed that dependence on environmental factors, such as temperature and soil moisture affected the *Fusarium* species. By the comparisons of all isolates it was concluded that they were not similar with each other at species level but show similarity at genus level. So it has been resulted that *Fusarium* species have changed their genetic material with passage of time for their survival. It can be concluded that morphologically similar strains of the same fungal species may show genetic variations so by this study species genotypes were identified which add knowledge about *Fusarium* species genotypes in Pakistan and this work helps to understand the genetic mechanisms for the efficient breeding programs to breed the resistant cultivars of different crops.

Poster #104

Antimicrobial, antioxidant and anticancer studies of *Bacillus cereus* and *Bacillus pumilus* metabolites isolated from soil samples of Malnad region of Shimoga, Karnataka, India.

THIPPESWAMY BASAIAH

North Carolina State University, Raleigh, NC USA and Kuvempu University, Karnataka India

Vijaya Kumar M L

National College of Pharmacy, Karnataka, India

Soil samples were screened for microorganism with antibiotic production potential against human pathogens. The metabolites of *Bacillus cereus* (BC-2) and *Bacillus pumilus* (BP-2) were subjected to successive solvent extractions and the test samples were prepared. The test samples were evaluated for antimicrobial, antioxidant and anticancer activity. Antimicrobial activity was studied by disc diffusion method. Metabolites of both the organisms showed good antibacterial activity but insignificant antifungal activity. During Minimum Inhibitory Concentration studies the test samples BC-2 and BP-2 exhibited MIC at 125 µg/ml against *S. typhi* and *K. pneumoniae* respectively. Antioxidant activity was studied by DPPH and ABTS radical scavenging assay. Metabolites of both bacteria exhibited significant antioxidant activity by exhibiting 97% and 88.5% radical scavenging by ABTS method for BP-2 and BC-2 with IC50 values at 16.2±1.17 µg/ml and 55.12±2.51 µg/ml respectively. The metabolites showed comparatively less radical scavenging activity by DPPH assay. Cytotoxicity study was carried on normal human liver cell lines and 2 cancer cell lines by MTT assay. The metabolite of BC-2 exhibited cytotoxicity on cancer cell lines. The two fractions were further screened for anticancer activity by nuclear staining studies and DNA fragmentation analysis on HepG2 cell lines. Both the fractions demonstrated significant activity by membrane blebbing during nuclear staining and damaged DNA patterns during DNA fragmentation analysis. The metabolites of BP-2 had toxic effect against both, cancer cells and normal cell lines.

Poster #105

Characterizing bacterial and fungal communities associated with plant drought tolerance

Chiliang Chen, Jeffrey Williams, Bo Xie, GWYN BEATTIE

Iowa State University, Ames, IA USA

Shulin Yang

Guizhou Normal University, Guiyang, Guizhou China

Water limitation is a major problem for crop production worldwide and is increasing in areas due to climate change and rising population straining water resources. Although plant microbes are known that enhance drought stress tolerance, we are applying a systematic approach to begin to associate microbial community members with plant tolerance to water deficits. We provided plants the opportunity to enrich for beneficial communities during successive cycles of growth under water-limited conditions and identified compositional shifts by comparing communities under water-limited and water-replete conditions and during successive growth cycles under each condition. Soil from a soybean field with a drought history was used to grow one bean and two soybean lines under low and high water conditions in four sequential 30-day growth cycles. Plants subjected to drought stress exhibited increased water use efficiency during the late cycles, consistent with a potential enrichment for beneficial microbes. Total community DNA from the bulk soil and the ectorhizosphere and endorhizosphere of each of four replicate plants was subjected to amplicon sequencing that targeted 16S rRNA and ITS sequences for bacteria and fungi, respectively, using a mixture of genomic DNA from 20 bacterial species containing staggered rRNA operon counts for verification of the bioinformatics pipeline. The drought treatment strongly favored bacterial (*Glycomyces*, *Streptomyces* and *Dyadobacter*) and fungal (*Fusarium*) genera in the endorhizosphere, while decreasing mycorrhizal fungi and some proteobacteria. These drought stress-associated shifts were consistent across all three plant genotypes, illustrating reproducible community development patterns and potential members contributing to drought stress tolerance.

Poster #106

***Poa annua* putting green turf exhibits widespread microbial diversity under high-input fertility regimes**

LISA A. BEIRN, James W. Hempfling, Charles J. Schmid, James A. Murphy, Bruce B. Clarke

Rutgers University, New Brunswick, NJ USA

Jo Anne Crouch

USDA-ARS, Beltsville, MD USA

Putting greens are the most intensely managed sites on golf courses. Fertilizer applications are commonplace, but little is known about their impact on the rhizosphere microbial community. We examined the effects of nitrogen (N) and potassium (K) on microbial communities in *Poa annua* putting green turf. Soil cores were sampled from two separate field studies. Field 1K plots received either 132 kg N ha⁻¹ yr⁻¹, 200 kg K₂O ha⁻¹ yr⁻¹, or 132 kg N and 200 kg K₂O ha⁻¹ yr⁻¹. Field 2 N plots received 100 or 200 kg N ha⁻¹ yr⁻¹. Organism-specific DNA regions from fungi (ITS), bacteria and archaea (16s) were sequenced in multiplexed reactions on the Illumina MiSeq platform. Analysis using QIIME revealed 8.3 X 10⁵ OTUs from the 60 samples (4.1% archaea, 62.1% bacteria, 30.1% fungi). Microbial diversity was high, regardless of treatment. DCA plots showed the Field 1K samples clustered separately from the Field 2 N study. Significant differences were observed in community structure across treatments, but were limited to a few taxa. These data show that N and K treatments can affect the composition and structure of rhizosphere communities in *P. annua* turf, but that additional management practices, or turf age, may also impact microbial distribution. To eliminate these factors, a separate field study was initiated to examine five N treatments, applied at different rates/intervals over 12 months. This data will determine if a wider range of N applications, or seasonality, affect microbial communities in the rhizosphere.

Poster #107

Preference of fungal colonization on candidate cover crop species and implications for crop health

MARIA SOLEDAD BENITEZ, Wendy I. Taheri, R. Michael Lehman

USDA-ARS North Central Agricultural Research Laboratory, Brookings, SD USA

Agricultural management practices promoting crop diversification are known to also promote microbial biomass and activity. The choice of plant species to be included in a diversified cropping system relates to the added benefits provided to the cropping system. Previous research has shown that the use of cover crops increases the number of propagules and promotes colonization of arbuscular mycorrhizal (AM) fungi in agricultural systems. However the extent to which individual cover crop species can select beneficial plant-associated microbial populations, compared to potential pathogenic groups has not been measured. The fungal communities associated to roots of individual plant species commonly used as cover crops were assessed after growth in a diverse soil inoculum originating from a prairie remnant. Specificity was observed both for plant host species in respect to the AM fungi that they selected, and for the AM fungal taxa found colonizing one or a range of the cover crop species tested. Similar patterns of specificity are expected for plant pathogenic taxa and other endophytic fungi. The ratio of beneficial to pathogenic taxa colonizing a particular cover crop species could further document the benefits for its use in a particular cropping system. Finally, we will integrate information on how management practices are affecting both pathogenic and beneficial fungal populations in the soil in order to promote cash crop establishment and maintenance of soil health.

Poster #108

An evaluation of mitochondrial COI barcode pyrosequencing for estimating oomycete species diversity in asymptomatic environments

JAIME BLAIR, Steven Clipman, Lauren Coffua, S. Tyler Veterano, Jorge Mena-Ali

Franklin & Marshall College, Lancaster, PA USA

Next-generation sequencing technologies are revealing a more complete picture of biological diversity within various environments and are posed to fundamentally challenge our current understanding of species interactions. However, in order to maximize the potential of this new resource, appropriate molecular loci need to be chosen and vetted to facilitate comparison across studies. Here we evaluate the use of the mitochondrial COI barcode locus for estimating oomycete diversity in both soil and aquatic habitats via pyrosequencing. COI has several advantages over other markers, namely the ITS and LSU ribosomal RNA regions, used in studies of fungal and microbial eukaryote diversity; because COI encodes a translated product, sequencing errors such as indels can easily be detected. In addition, the conservation of COI allows for more robust sequence alignment and phylogenetic analysis across greater evolutionary distances, while still retaining enough nucleotide variation for species-level identifications. Our pyrosequencing results recovered more than twice the number of oomycete species compared to our culture-based approach (58 versus 25 species, respectively); in addition, we were able to identify 40 well supported, phylogenetically novel lineages, some of which may represent new species. As with all sequenced-based approaches, a number of important biases must be considered in order to realize the full potential these new and exciting technologies bring to the study of phytobiomes.

Poster #109

Genomic requirements of endophytic symbionts providing long-term improvements in plant function

MOLLY CADLE-DAVIDSON, Gary Harman

Advanced Biological Marketing Inc., Geneva, NY USA

Existence of the phytobiome highlights the concept that no living organism operates on its own and the corollary that genomic functions of phytobiome organisms are interdependent. Natural selection has acted on microorganisms and plants such that the collective genome is what allows the phytobiome to respond, adapt, and thrive. Crop plant phytobiomes can be altered using agricultural inoculants with specific endophytic symbionts conferring improved disease resistance, plant vigor, abiotic stress resistance, and yield. These physiological changes are long-term and specific to the inoculant strains applied. Some of these interactions, such as rhizobia-mediated nodulation in legumes, are well characterized and the genomic requirements known. Other crop interactions involving *Trichoderma* or *Bacillus* are less well described with the main predictor being metabolite production. These deficiencies are largely due to lack of statistical power of the microbial populations evaluated. We have generated Illumina sequence data (min. 100X depth) for our collections of *Bacillus* and *Trichoderma*. These organisms are represented by dramatically different population structures and different analyses are being employed: *Bacillus* requiring association mapping strategies and *Trichoderma* having near-isogenic relationships. Our populations are characterized for a growing number of microbial and agronomic (expressed in the plant upon root colonization) traits, totaling more than 24 at the time of this writing. The combination of comprehensive phenotypic data, informative population structures, good sequencing depth and existing excellent genomic resources is allowing us to identify not only the genes critical to evolution of the phytobiome but also for optimization of microbe-mediated crop performance enhancements.

Poster #110

Exploiting Plant-microbe Interactions to Enable Sustainable Growth of Plants

ROMY CHAKRABORTY, Marcus Schicklberger, Stefan Jenkins, Trent Northen, Dominique Loque

Lawrence Berkeley National Laboratory, Berkeley, California USA

It is well known that plant associated microbes can significantly influence nutrient availability. Diazotrophic endophytes are known to fix atmospheric N to bioavailable forms of N in diverse plants. Our knowledge on the recruitment, maintenance, interaction of diazotrophic endophytes with host plant is very limited. Understanding the detailed mechanism of these processes is imperative, and could have significant impact on growing plant sustainably. Our research focuses on investigating interactions between plants and such endophytic diazotrophs, we present results from our investigation with endophytic diazotrophs associated with Tobacco (*Nicotiana tabacum*) and Switchgrass (*Panicum virgatum*). We successfully isolated several endophytic strains from the roots and leaves of Tobacco and Switchgrass plants and confirmed N-fixation using the well-established Acetylene Reduction Assay as well as by the detection of *nifH* genes. All the isolates readily utilized sugars and simple organic acids as Carbon source, and have a temperature optima of 25°C. Seedlings inoculated with a diazotrophic endophyte, strain R1Gly, enhanced plant biomass. In order to identify metabolites that potentially attract N-fixing bacteria to plants, diazotrophic isolate *Azospirillum* strain R1C was grown in root exudates collected from hydroponically grown plants, and analyzed for substrate uptake/release. Detailed knowledge and understanding of such interactions between diazotrophic endophytes and host plant will allow us to design robust strategies to enhance plant biomass using microbial N-fixation and will ultimately allow for decreased dependency on fertilizers.

Poster #111

Effect of Downy Mildew and Biofungicides on the Phyllosphere of Spinach

ROBIN CHOUDHURY, Nilesh Maharaj, Johan Leveau, Neil McRoberts

University of California, Davis, CA USA

Steven Koike

University of California Cooperative Extension Monterey County, Salinas, CA USA

The most serious threat to spinach production in California is downy mildew, caused by the obligate biotrophic pathogen *Peronospora effusa*. Spinach downy mildew affects all growing regions of California and is especially damaging to fresh market spinach. To attempt to manage the disease, many growers rely heavily on major-gene resistance and fungicides. Organic growers rely primarily on resistant varieties, although recent work suggests that there are efficacious biofungicides. Relatively little is known about how biofungicides and disease impact the phyllosphere microbiota of spinach. In this study, we examined the effects of four fungicides (Zampro, Cueva, Actinovate AG, and Serenade Optimum) and an untreated control on the bacterial and fungal communities of the spinach phyllosphere by sequencing the 16S rDNA V4 and ITS1 regions, respectively. In addition to this work, we also examined the effect of different levels of downy mildew disease (0%, 10%, and 50%) on the phyllosphere of untreated field grown spinach plants. Samples are being sequenced using the Illumina MiSeq platform and will be analyzed using QIIME. Results will be discussed at the poster.

Poster #112

The fungal community associated with orchid soils in Sweden

MARC CUBETA

North Carolina State University, Raleigh, NC USA

Shahid Mahmood, Roger Finlay, Bjorn Lindhal, Jan Stenlid

Swedish University of Agricultural Sciences, Uppsala Sweden

Fungi classified as Rhizoctonia have been intensively studied because of their economic importance as pathogens of agricultural, forestry and ornamental species of plants. However, Rhizoctonia fungi can also establish beneficial symbiotic associations with orchids to promote seed germination and seedling growth. These fungi can also associate with liverworts and mosses without inducing any apparent harm. In this study, soil samples were collected from eight geographic locations in central Sweden where orchid seedlings of *Cypripedium calceolus*, *Dactylorhiza incarnata*, *Dactylorhiza maculate*, *Epipactis palustris*, *Listera ovata* or *Plantathera bifolia* were growing. Total genomic DNA was extracted from the soil samples and the resulting amplicons were subjected to high throughput pyrosequencing-based methods and a bioinformatics platform developed by Lindhal and colleagues. To identify operational taxonomic units (OTUs) and to characterize members of the soil fungal community, the generated sequences were subjected to RDP classifier using UNITE fungal ITS reference database. The number of fungal OTUs sampled at each site ranged from 222 to 863. Fourteen OTUs were identified from all locations sampled, with five taxa tentatively identified to the species level (*Exophiala equina*, *Mortierella amoeboides*, *Operculomyces laminatus*, *Phoma brasiliensis*, and *Tetracladium furcatum*). Taxa typically forming symbiotic associations with photosynthetic terrestrial orchid seeds and seedlings (i.e., *Ceratobasidium*, *Sebacina*, *Thanatephorus*, and *Tulasnella*=*Epulorhiza*) occurred in low frequency (>1%) across all samples, except for *Epulorhiza* sp. MO41 and two species of *Sebacina* at two locations where seedlings of *C. calceolus* and *L. ovata* were present, respectively.

Poster #113

The Effects of Fungicides on Phyllosphere Microbes

JOSEPH DOHERTY, Joseph Roberts

University of Maryland, College Park, MD USA

Megan Botti-Marino

Michigan State University, East Lansing, MI USA

David Ritchie, James Kerns

North Carolina State University, Raleigh, North Carolina USA

Golf course putting greens are prone to a myriad of diseases and fungicides are periodically applied to maintain turf quality and playability. The effect of these periodic fungicides applications on the phyllosphere microbial community has not been well studied. The aim of this research project was to determine the impact of commonly applied fungicides (chlorothalonil, fluxapyroxad, fosetyl-Al, fluazinam, pyraclostrobin, and none) on phyllosphere microflora of an 'A-1' creeping bentgrass putting green. Treatments were applied every 14 d throughout the summer at label rates. Turfgrass phyllosphere samples were extracted from 4 random replicates five days after each application. Turf samples were homogenized in sterile diH₂O, diluted to 10⁻³ and 10⁻⁵ and plated on four separate media [actinomycete isolation agar, acidified potato dextrose agar (APDA), nutrient agar + 1% sucrose (NAS), and King's B (fluorescent pseudomonas isolation agar)]. Colony forming units (CFUs) of all microorganisms were counted 2 and 7 d after culture and normalized to the weight of the sample. Overall, fungi, bacteria, and actinomycete populations declined after initial fungicide applications, but actinomycete and bacteria populations rebounded after the third fungicide application. Chlorothalonil, fluxapyroxad, and pyraclostrobin lowered populations of fungi, and fluazinam lowered populations of actinomycetes and bacteria when compared to the control. Future studies that incorporate metagenomic analyses will improve our knowledge of non-target effects of fungicides applied to turfgrasses.

Poster #114

Phytophagous hemipteran insect honeydew as a growth medium for plant-associated *Salmonella enterica*

JP DUNDORE-ARIAS  R Groves, JD Barak

University of Wisconsin, Madison, WI USA

Phytophagous hemipteran insects could interact with *Salmonella enterica* in agricultural environments and have been shown to influence the persistence and distribution of the pathogen on plants. To understand the mechanisms behind this interaction, we examined the potential for honeydew to function as a growth medium for *S. enterica*. When feeding on plant phloem sap, hemipteran insects excrete large amounts of honeydew on the leaf surface, suggesting that this sugar-rich excretory product could influence the ability of *S. enterica* to survive on plants. Honeydew was collected from adult *Macrostelus quadrilineatus* and *Myzus persicae* fed on a glucose or sucrose liquid diet or leaves. Subsequently, *S. enterica* replication was analyzed by comparing populations from cultures inoculated in honeydew, sterile water or sugar solutions. Higher populations of *S. enterica* were found in honeydew excreted by both hemipteran species, compared to populations in sugar solutions or water, regardless of the type of insect diet provided. Additionally, we examined *S. enterica* populations on leaves exposed to *M. persicae* containing a visible film of honeydew. Leaves with high honeydew deposition supported higher *S. enterica* populations than leaves without honeydew. Thus,

presence of hemipteran insect and their excreta on plants may enhance the persistence of epiphytic populations of *S. enterica*. These results expand our limited knowledge of the relationship between human pathogens and phytophagous insects in association with plants, and highlight how complex multi-trophic interactions in the phytobiome could pose a risk to agricultural production, food safety, and human health.

Poster #115

Characterization of the Citrus phytobiome: Identifying endophytic microbes with potential to improve tolerance to plant disease

NICHOLE GINNAN, Tyler Dang, Paul Ruegger, James Borneman, Philippe Rolshausen, Georgios Vidalakis, M. Caroline Roper
University of California, Riverside, CA USA

There is compelling evidence that the health of an animal or plant is linked to the structure and activities of its associated microbiome. The goal of this project is to characterize the endophytic phytobiome in citrus grown in different citriculture areas in the United States. We have isolated and identified the culturable microbes from phloem-rich bark peels collected from two distinct citrus production areas in southern and central California. Notably, our study included the 142 year-old Parent Washington Navel Orange tree in Riverside, California. We isolated both fungi and bacteria and identified them based on the sequences of their 16S rRNA gene for bacteria and the ITS region of the ribosomal operon from fungi. A culture-independent analysis of the endophytic citrus phytobiome using an Illumina MiSeq platform is also underway. We hypothesize that the citrus endophytes, particularly phloem-inhabiting microorganisms, and their associated metabolomes can be used to reduce or prevent colonization of citrus pathogens, such as *Candidatus Liberibacter asiaticus*, due to their shared ecological niche of the phloem. We are currently experimenting with techniques designed to transfer the endophytic citrus phytobiome from a donor tree to a recipient host tree. We envision that such methods can be used to transfer beneficial phytobiomes from a donor tolerant or disease resistant tree to a recipient host tree to combat citrus diseases.

Poster #116

Effects of Maize Genotype and Nitrogen Fertilization on the Soil and Plant Microbiome

L. J. HALVERSON, C. Chen, G. J. Smith, A. M. Holmes, G. Bay

Iowa State University, Ames, IA USA

We examined how maize genotype and nitrogen (N)-fertilization levels influence soil, rhizosphere, and endophytic bacterial communities. Our approach was to grow the intermated B73 x Mo17 (IBMsyn10) maize mapping population lines in rhizotrons filled with soil obtained from a long-term study with >12 year history of variable (0, 60, 120, and 240 lb N /acre) fertilization inputs. After four to six weeks of growth under varying N inputs, samples were collected from bulk soil, rhizosphere (defined as the microbes intimately associated with roots but removed by washing) and the endophytic community of the entire root system. Total DNA was isolated from these samples for amplicon sequencing of the V3-4 region of the 16S rRNA gene using the Illumina MiSeq platform. Consistent with previous observations, our data indicate that the bulk soil, rhizosphere and endophytic compartments are comprised of distinct prokaryotic communities. Within each compartment, however, we observed that N inputs affect prokaryotic profiles: samples from low N inputs (0 and 60) shared similar bacterial profiles, but are distinct from those collected from high N inputs (120 and 240). Interestingly, we observed that certain maize genotypes exhibited distinct rhizosphere and endophytic bacterial profiles under the same N-fertilization level. Additionally, quantitative PCR assessments revealed that N fertilization and maize genotype influenced the abundance of ammonia oxidizing -archaea (AOA) and -bacteria (AOB) in soil and the rhizosphere. Our findings should enable dissection of plant-microbe interactions contributing to maize health and environmental influences on those communities, including the N transformations they mediate.

Poster #117

Comparison of commercially available soil DNA extraction kits on soil treated with anaerobic soil disinfestation

JASON HONG, Nancy Kokalis-Burelle, Erin Roskopf

USDA-ARS, US Horticultural Research Laboratory, Fort Pierce, Florida USA

Kendall Martin

William Paterson University, Wayne, New Jersey USA

David Butler

University of Tennessee, Knoxville, Tennessee USA

Anaerobic soil disinfestation (ASD) is a biologically-based alternative to pre-plant soil fumigation for managing weeds and plant pathogenic microorganisms. ASD consists of working in soil amendments, covering soil with polyethylene mulch, and saturating the soil to create an anaerobic environment. Treated soil becomes favorable for anaerobic and facultative anaerobic microorganisms, which produce antimicrobial compounds. A molecular approach was taken to identify which bacterial populations are important for ASD. Microbial DNA was extracted from ASD-treated and non-treated soils. Five commercially-available microbial DNA extraction

kits were used to extract the same samples. The methods for two of the kits were modified from the manufacturer's protocol and included additional purification steps. Thus, a total of seven different methods were compared based on quantity of extracted DNA, quality of DNA, PCR amplification, amplicon concentration, amplification of a specific target population, metagenomic sequencing, and biodiversity measurements including total bacterial populations, Shannon indices, and evenness. Comparison of the post-treated ASD and untreated soil samples showed that the biodiversity was greater for the untreated soil for all of the methods except two. Significant differences in DNA yield, quality, and biodiversity were dependent on the DNA extraction method used. Thus indicating the importance that all samples within a study be prepared by the same method, and the need for attention when cross-comparing studies that used different methods.

Poster #118

Genetic Improvements in Recombinant Protein Accumulation in Maize Embryos

ELIZABETH E. HOOD, Thomas (Keat) Teoh

Arkansas State University, Biosciences Institute, Jonesboro, Arkansas USA

John A. Howard

Applied Biotechnology Institute San Luis Obispo CA USA

Our long-term goal is to use a plant seed system as a biofactory for producing proteins for industrial or pharmaceutical applications. Although this is an applied project goal, the pathway to success has generated many interesting basic scientific principles, questions and challenges. Our research group has observed the phenomenon of improved transgene expression over generations using genetic selection in maize. These selections were performed for increasing transgenic protein accumulation in seed, and results have been achieved on as many as 15 proteins in several genetic backgrounds. The selections have been empirical; we have screened thousands of individual ears over multiple generations for these increases. Examples include avidin (Hood and Woodard, 2006), LT-B (Streatfield et al., 2002), laccase (Hood et al., 2003) and cellulase (Hood et al., 2012). We seek to understand the genetic factors that control increased recombinant protein accumulation in maize using a transgenic line expressing a cellulase gene. Our system offers a powerful tool to selectively analyze gene expression from native promoters with genes that can be easily distinguished from native genes. We have established near-isogenic recombinant inbred lines derived from a single parent individual that express the CBH I gene at high and low levels in a single generation. These lines are being compared for differential gene expression through RNA-seq, and comparing them also to the non-transgenic control. Because of the embryo-preference of the promoter, embryos are sampled at 15, 21, and 24 days after pollination during storage protein accumulation. Preliminary results will be discussed.

Poster #119

The Sugarcane Microbiome: A Key to Crop Sustainability?

JUAN IMPERIAL, Beatriz Jorrín

Centro de Biotecnología y Genómica de Plantas (CBGP), UPM, Madrid Spain

Rafael Soares Correa de Souza, Paulo Arruda

Centro de Biologia Molecular e Engenharia Genética (CBMEG), UNICAMP, Campinas Brazil

Sugarcane (*Saccharum* L.) is a major crop that satisfies most of the world's sugar needs. Brazil is the world's first sugarcane and sugar producer, and 70% of its sugarcane is grown in the South-East region, where yields of 70-150 Tm biomass per hectare are consistently obtained with minimum fertilization and with no apparent depletion of soil nutrients, nitrogen in particular. These observations led Joanna Döbereiner to hypothesize in the 1970s that sugarcane plants must establish associations with nitrogen-fixing microorganisms and that these associations are critical for the crop's sustainability. That sugarcane can fix nitrogen has been repeatedly demonstrated in field experiments, and Döbereiner's work resulted in the isolation and characterization of new and diverse endophytic diazotrophs, such as *Azospirillum*, *Herbaspirillum*, and *Gluconoacetobacter*. However, it is unclear whether these organisms, present in sugarcane in small numbers, are responsible for the nitrogen fixation observed at the agronomic level. In order to locate and identify those microorganisms contributing to the high sustainability of sugarcane, we have undertaken the complete characterization of the microbiome of the different compartments (root, culm, root-culm interface, leaves) from highly sustainable sugarcane varieties, including bacteria and fungi present in, on, and around the plant, first through 16S, 18S and ITS amplicon metagenomic analysis of these compartments, and later through shotgun metagenomics. This characterization is ongoing, and the first results will be presented. Supported by research contracts and with the collaboration of Repsol (Spain) and Repsol-Sinopec (Brazil).

Poster #120

Development of an Effective Phytoremedial Technology for Metal Contaminated Calcareous Soils

SHAZIA IRAM, Shazia Akhtar

Fatima Jinnah Women University Rawalpindi, Punjab Pakistan

In present study peri-urban agricultural areas of Punjab, Pakistan were selected. Soil samples irrigated with municipal and industrial wastewater were collected from two depths 0-15 and 15-30". These soil samples; wastewater and crops/vegetable samples collected from study areas were analyzed for heavy metals contamination. Soil micro fungi were also isolated by standard methods. Copper, lead, cadmium and Chromium were accumulated more than recommended permissible limits in the studied areas. Natural, biological and chemically enhanced phytoextraction potentials of maize (*Zea mays L.*) and mustard (*Brassica campstrus*) were explored by growing them on two contaminated soils of for 75 days. Soils were amended with varying amounts of DTPA at 0, 1.25, 2.5, and 5.0 mM kg⁻¹ soil and inoculums of three fungal species (*Aspergillus niger*, *Aspergillus fumigatus* and *Aspergillus flavus*) to enhance metal solubility and availability. In pot experiment under green house, addition of fungi and DTPA significantly increased the Cu, Pb, Cr and Cd concentrations in roots and shoots and also increased uptake, Bioconcentration factor, Phytoextraction rate and Extraction efficiency over the control. Overall *A. fumigates* presented good results in increasing biomass production of maize and mustard plant in both Gujranwala and Lahore soils. Post harvest metals contents in soil were analyzed and *A. flavus* and *A. fumigates* showed better behavior in making metals available by their solubilizing efficiency. A phytoremediation model was developed based on generic data for the reclamation the metals contaminated calcareous soils of Punjab.

Poster #121

Interaction of *Phytophthora erythroseptica* with Soil Microbes and Host Plants Mediated by Signaling Molecules

HELEN JIANG, Jianjun Hao

University of Maine, Orono, ME USA

Phytophthora erythroseptica causes pink rot of potato. Its flagellate zoospores serve as inocula for host infection. It was shown that the zoosporic behavior, such as germination and host infection of *P. erythroseptica*, was dependent on the zoosporic density, or regulated by quorum sensing. To elucidate the regulation, laboratory and greenhouse trials were conducted. Zoosporic extracellular products (ZEPs) of *P. erythroseptica* at various concentration were collected by filtration, which was used to treat *P. erythroseptica* zoospores. As a result, a single zoospore did not germinate or infect the host, but did so if treated with ZEP from a highly concentrated zoospore suspension, suggesting ZEP contains signaling molecules regulating the zoosporic behavior. The functional molecules or homologs were not only obtained from the same species of *P. erythroseptica*, but also its close relatives. Furthermore, they were from host plants, bacteria, and microbe-enriched soil. Test microorganisms from different taxa induced zoosporic germination of *P. erythroseptica* as well root exudates of potato plants. This indicated that the pathogen is affected by many biological factors in the pathosystem. In analyzing the chemicals of ZEP, several compounds have been considered as candidates, but needs further investigation.

Poster #122

Engineering a Synthetic Genetic Toggle Switch in Plants for Bioenergy Applications

TESSEMA KASSAW, Christopher Zalewski, Katherine Schaumberg, Wenlong Xu, Camila Saldanha, Mauricio Antunes, Ashok Prasad, June Medford

Colorado State University, Fort Collins, CO USA

Striking progress has been achieved in improving biofuel production to guarantee reliable source of clean energy and address global climate change and economic sustainability for the past few years. Since the current production of biofuels relies heavily on edible crop plants, genetic improvement of alternate bioenergy plants is compulsory. Sorghum is an excellent alternative to meet the needs for next-generation biofuels, mainly due to its drought tolerance, adaptability to diverse growing conditions, low nitrogen fertilizer requirements, high biomass production and utilizes the existing infrastructure from grain crops. However, the key limitation for improvement of bioenergy crops, including sorghum, is the ability to rapidly introduce new traits via genetic transformation with superior efficiency. To address this limitation, we are engineering synthetic genetic circuits that enable plants to switch between embryonic and vegetative states. The circuit uses synthetic repressor-repressible promoter pairs that are quantitatively balanced and can be controlled by external inducers to toggle between two stable output states (ON vs. OFF). We have designed and quantitatively characterized the behavior of a series of these pairs transiently in protoplasts. The kinetic parameters derived from these assays were used to build a computational model of the plant genetic toggle switch, and transgenic plants containing bistable genetic circuits were generated. This genetically encoded "toggle-switch" will allow researchers of any skill level to develop the materials needed for routine, controlled and highly predictable transformation. By modifying the output of these toggle switches, we hope to increase the engineering potential of sorghum for bioenergy applications.

Poster #123

Phytobiomic basis of plant disease resistance

JIHYUN F. KIM, Min-Jung Kwak

Yonsei University, Seoul, Republic of Korea

Seon-Woo Lee

Dong-A University, Busan, Republic of Korea

The three elements of the disease triangle for an epidemic constitute a susceptible plant host, a virulent pathogen, and a favorable environment such as moisture and temperature. Among the environmental factors, biotic factors, e.g., commensal microbiota that inhabits the plant, may significantly contribute to disease development. To test this possibility, we initiated a whole metagenomic analysis of microbial communities in the rhizosphere of two tomato cultivars, Hawaii 7996 and Moneymaker, which are either resistant or susceptible to a bacterial wilt caused by *Ralstonia solanacearum*. Taxonomic comparison of the recruited 16S rDNA sequences demonstrated that relative abundance of the class *Flavobacteria* is higher in the rhizosphere of Hawaii 7996 than that of Moneymaker. On the other hand, relative abundance of *Bacilli* and *Betaproteobacteria* were higher in the rhizosphere of Moneymaker. These tendencies cohered with the results from the reference genome-guided analysis. To compare the gene contents, *de novo* assembly and gene prediction followed by COG and Subsystem assignments were conducted. When the scaffolds were sorted according to the bacterial phyla, scaffolds assigned to *Bacteroidetes* had higher fold-coverage in Hawaii 7996 than Moneymaker. Paired-end reads of the scaffolds of *Bacteroidetes* in Hawaii 7996 were extracted and re-assembled, to reveal the genome of an unclassified *Flavobacteraceae* bacterium. The genome had a higher proportion of genes related to the metabolism of inorganic molecules including iron. These results suggest that microbiome function in the rhizosphere of the resistant plant may play a pivotal role in suppressing the incidence or severity of the soil-borne disease.

Poster #124

Genetic diversity and abundance of cyanobacteria and associated microbes in agricultural runoff containment reservoirs

PING KONG, Chuanxue Hong

Virginia Tech, Virginia Beach, VA USA

Agricultural runoff containment reservoirs (ARCRs) are an emerging aquatic system of global significance to crop production, water and environmental sustainability. This study determined the algal diversity and abundance in this nutrient-rich aquatic system. Eight ARCRs of different nutrient loads at three ornamental crop nurseries and an adjacent creek in eastern and central Virginia were surveyed. Water samples were taken during spring and their algal diversity was typed using molecular methods. DNA was amplified with cyanobacteria-specific primers based on the 16S rRNA gene then used for clone library construction. A total of 856 high quality clone sequences were generated in nine clone libraries and they were identified to 209 operational taxonomic units (OTUs). Among these were 353 clones in 53 OTUs of cyanobacteria dominated by *Synechococcus*, 62 OTUs (389 clones) of eukaryotic phytoplankton including green algae, algae, and algae-like organisms, and 93 OTUs (114 clones) of other bacteria. Cyanobacteria dominated sedimentation reservoirs fed with agricultural runoff, while other bacteria dominated the undisturbed creek. Transition and retention reservoirs indirectly receiving runoff via a sedimentation reservoir were dominated by eukaryotic phytoplankton. The greatest microbial diversity was observed in the creek and the least in the sedimentation reservoirs. These results are foundational for understanding of recycled water quality dynamics and developing agricultural runoff into a good quality alternative water resource. They also highlight the importance of ARCRs to conserving microbial diversity in natural aquatic systems. This study provided numerous leads to converting ARCRs into bioreactors for potential agricultural, biotechnological and environmental applications.

Poster #125

Differential gene expression studies and protein profiling in *Phyllanthus fraternus* and *Daucus carota*

Sukhendu Bikash Ghosh

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra India

DINESH KUMAR

Singhania University Rajasthan, Pachari Bari, Rajasthan India

In the present study, we have studied the differential gene expression pattern of the potential candidate genes of the corresponding proteins which has been identified in carrot callus culture in response to the MS media and MS supplemented with 2, 4-D in *Phyllanthus fraternus* callus culture by RT-PCR and also identified the differentially expressed proteins in the suspension culture using MALDI-TOF/MS. *Phyllanthus fraternus* is an important medicinal plant but has not been very well characterized at the genomic and proteomic level. We have identified 10kDa protein in 15 days old suspension culture which is expressed in liquid MS modified media. This protein is ubiquitin like protein and possibly has a role in protein turn over and the ability in mediating protein-protein interactions. We further recommend the study of molecular principles of hormonal regulations, mechanisms determining the specificity of hormonal pathways, targeted manipulation of the development of crop plants to obtain desired production properties,

control of biomass production under extreme environmental conditions, development of phyto-remediation strategies, application of metabolomics in functional genomics, diagnostic and plant secondary metabolism.

Poster #126

The effects of plant genotype and local soil on diazotrophs associated with *Miscanthus*

DONGFANG LI  Angela Kent

University of Illinois, Urbana, IL USA

Miscanthus species are promising biofuel feedstock candidates that have great capacity adapting to various tropical and subtropical climate and soil conditions. Nitrogen is often the most limiting nutrient for plants in the agricultural system. Previous N cycle modeling and 15N dilution experiments suggested that diazotrophs associated with *M. xgiganteus* contribute nitrogen to plant growth and sustainability. The community composition of bacteria associated with the plant is under the influence of biotic factors such as plant genotypes and local soil bacteria. Therefore, the positive interaction between *Miscanthus* and diazotrophs may not remain effective across locations and different genotypes. To better understand *Miscanthus*-diazotrophs association, the effects of *Miscanthus* genotype and soil on the associated diazotrophs were examined with field and greenhouse experiments with factorial design. Firstly, genetically identical *M. xgiganteus* rhizomes cultivated in four field sites were collected for microbial analysis after 3 years of cultivation. Results from this study showed that when the same *Miscanthus* species was planted in different geographic regions, the endophytic compartment of these plants tended to harbor similar diazotroph communities, even though, the rhizosphere soil diazotroph community pools are distinct. Secondly, a common garden experiment using four *Miscanthus* genotypes and two soil sources were conducted. When exposed to the same soil bacterial community pool, closely related *Miscanthus* species tend to harbor dissimilar diazotroph communities in the endophytic compartment. To conclude, the positive interactions between *Miscanthus* and diazotrophs depend on both site condition and *Miscanthus* genotype.

Poster #127

Preliminary Study on Endophytic Microbiome Associated with Suppression of Soybean Charcoal Rot Disease

Sonya Baird, Beth Stokes, Richard Baird, SHI-EN LU

Mississippi State University, Mississippi State, MS USA

Charcoal rot caused by the fungus *Macrophomina phaseolina* is the most economically important soilborne disease of many agricultural crops in the United States. While charcoal rot causes high mortality, it is common to find asymptomatic soybean plants with similar genetic makeup and grown in the same environmental conditions within disease patches in fields. We hypothesized that endophytic bacteria and/or fungi play a critical role in plant resistance to the disease, through either biological priming of the plant or direct antagonism against the pathogen. To test the hypothesis, microbial populations and composition were preliminarily investigated using both culture- dependent and –independent methods. More than 40 bacterial species were obtained from soybean tissues, which include *Bacillus* spp., *Pseudomonas* spp., and *Curtobacterium* spp., and their richness in both diseased and resistant plants were different. Similarly, some endophytic fungal isolates that belong to *Trichoderma harzianum*, *Fusarium solani* and *Verticillium lecanii*, which possess antagonistic activities, were recovered from the resistant plants. In addition, plate bioassays revealed that some endophytic fungi possess antagonistic activities against *M. phaseolina*. Currently, analysis of microbial communities of the resistant and diseased plants using Illumina sequencing to identify organisms enriched in the healthy plants is in under way. The research will provide solid data to better understand three-party interactions among endophytic bacteria or fungi, the fungal pathogen, and the host plant.

Poster #128

Genetics of plant-microbe interactions in natural populations

DEREK LUNDBERG  Sonja Kersten, Julian Regalado, Dino Jolic, Gautam Shirsekar, Detlef Weigle

Max Planck Campus, Tübingen Germany

While many one-to-one plant-microbe interactions have been studied in detail in lab experiments, nature exposes plants to large communities of microbes that are also interacting amongst themselves. Many of these microbes evade or tolerate the plant immune system and colonize leaves, posing a risk to the plant. Because maintaining an immune system is costly, successful plants streamline their defense capabilities. In *Arabidopsis thaliana*, many putative pathogen resistance genes, as well as pattern recognition receptors that recognize microbial molecules, are uncharacterized. These immune gene families are extremely variable, with great genetic variation maintained even within single field sites. The functional consequences of these immune gene repertoires in wild plants are essentially unknown. To explore the evolutionary genetics of plant-microbe interactions in *A. thaliana* in a wild setting, I am beginning a project to leverage high-throughput sequencing and classification methods to genotype and quantify both eukaryotic and prokaryotic phyllosphere microbes on a sub- species scale, and associate them with plant loci. I will present forthcoming results of shotgun metagenomic analysis of rosettes from 92 wild plants from two field sites in Germany, and will comment on feasibility of brute force sequencing vs. microbial enrichment methods. I will also describe an inexpensive, non-toxic, and automation-friendly metagenomic DNA prep for plant material at a fraction of the price of common kits.

Poster #129

Impact of disease management on root and soil microbiota of field-grown processing tomatoes

NILESH MAHARAJ, R. Mike Davis, Johan Leveau

University of California, Davis, CA USA

Eugene Miyao

University of California, Cooperative Extension, Woodland, CA USA

Over a three-year period from 2011 to 2013, we conducted six field trials in Yolo and Solano County, CA, processing tomato fields to evaluate the impact of drip-injected chemical and biological fungicides and soil-incorporated composted poultry manures on rhizosphere and bulk soil microbiota. Treatments included: Vapam (active ingredient metam sodium), Quadris (azoxystrobin), Ridomil Gold SL (mefenoxam), Tenet (*Trichoderma asperellum* and *T. gamsii*), SoilGard (*Trichoderma virens*), Serenade Soil (*Bacillus subtilis*), Actinovate (*Streptomyces lydicus*), Soil System I (beneficial bacteria blend), and composted poultry manure. We profiled the bacterial and fungal communities of around 800 samples by Illumina sequencing of amplified bacterial 16s rDNA V4 regions and fungal ITS 1 regions. We did not detect significant differences in the bulk and rhizosphere soil microbiota between treatments, with the exception of composted poultry manure at one of the field sites, where there were significantly higher relative abundances of bacteria from the genera *Achromobacter*, *Cellulosimicrobium*, *Mycoplana*, and *Sphingobacterium*. The root microbiota were enriched with fungal pathogens of tomato, including *Pyrenochaeta lycopersici*, *Plectosphaerella cucumerina*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Colletotrichum coccodes*. DNA-based, high throughput analysis of soil-root microbial community structure has the potential to reveal novel constituents and modifiers of the disease triangle.

Poster #130

Soybean phyllosphere microbiome reveals diverse microbial population

SHIN-YI MARZANO, Houston Hobbs

University of Illinois, Urbana, IL USA

Glen Hartman, Leslie Domier

USDA-ARS, Urbana, IL USA

Little is known about the microbial composition of the soybean phyllosphere, which could contain microorganisms that are either directly or indirectly antagonistic to economically important fungal plant pathogens. In this study, metatranscriptomes were compared from soybean leaves collected from soybean fields at multiple locations in Illinois, USA in 2008, 2009, 2010, and 2014. Metatranscriptomic analyses produce information on community composition as well as the relative abundance of actively transcribed genes that could impact plant-microbiome interactions. Each year, total RNA was extracted from the leaf samples, pooled, and depleted of ribosomal RNAs. RNA-Seq libraries were prepared and analyzed by paired-end high throughput sequencing. Individual sequence reads were aligned to a subset of the NCBI nonredundant data base using DIAMOND, parsed and processed using MEGAN5. Based on the normalized reads assigned to kingdoms, bacteria represented from 1% to 4% of the total aligned reads. An average of 2.8% of the reads aligned to virus sequences. The remaining reads were mostly eukaryotic in origin. Among the bacterial reads, 48% on average belonged to Cyanobacteria, followed by 39% to Proteobacteria, 9% to Firmicutes and 1.1% to Actinobacteria. Among the eukaryotic reads, 95% were assigned to plants (soybean), 1.1% to Ascomycota, followed by 0.3% to Arthropoda, specifically aphids, followed by less than 0.01% each to Basidiomycota and Oomycetes, and less than 0.01% to Nematoda. In future studies, we propose to further investigate the association between plant phenotypes and the composition of the phyllosphere transcriptome.

Poster #131

Belowground Plant Associations with Mycorrhizal Fungi and Their Influence on Phloem-Feeding Insects

ABHINAV KUMAR MAURYA  Susana Gomez

University of Northern Colorado, Greeley, Colorado USA

Arbuscular mycorrhizal fungi (AMF) represent an important component of the soil microbiome. More than 80% of land plants form beneficial associations with AMF, which provide essential nutrients (phosphorus and nitrogen) to the plant in exchange for carbohydrates. AMF alter the aboveground interactions between plants and insect herbivores such as aphids, as both organisms share the same plant host. Aphids are major agricultural pests because of their ability to transmit plant viruses; they have a wide host range, they are able to evade plant defenses, and their ability to develop insecticide resistance. This research involves interactions between barrel medic plants (*Medicago truncatula*) with the AM fungus *Glomus intraradices* and pea aphids (*Acyrtosiphon pisum*). It is aimed at quantifying the impact of tripartite interactions on each organism's performance to confirm whether AMF increase mycorrhiza-induced resistance against aphids. Our preliminary data showed that plant interactions with aphids and AMF operate in both directions: (i) at medium levels of root colonization by AMF (40-50% root-length colonized) there was no significant impact on the pea aphids' ability to feed, survive and reproduce, (ii) at high levels of root colonization by AMF (>70% colonization), there was a positive impact on pea aphid abundance when insects fed on well colonized plants. The gene expression analyses are underway, which may allow us to develop predictions that explain why certain mycorrhizal plants are more susceptible to aphids.

Poster #132

The Microbes of *Theobroma cacao* L. and Their Ability to Modulates Plant Gene Expression and Disease Resistance

SIELA N. MAXIMOVA, Mark J. Guiltinan

The Pennsylvania State University, University Park, PA USA

Luis C. Mejia, Allen E. Herre

Smithsonian Tropical Research Institute, Balboa, Ancon Republic of Panama

Plants in natural or agricultural settings typically host diverse bacterial and fungal microbiomes that can affect various plant functional traits such as resistance to abiotic and biotic stresses. Nonetheless, the individual species in these rich microbiomes are largely uncharacterized taxonomically, evolutionarily and at functional level. Specifically, mechanisms underlying endophyte effects on plant responses to biotic stresses such as pathogen infection is poorly understood. To address some of these questions, we conduct research on the interactions of *Theobroma cacao* L., a neotropical forest tree species, with its' dominant foliar fungal endophyte, *Colletotrichum tropicale* and two of its' pathogens, *Colletotrichum theobromicola* and *Phytophthora palmivora*. Inoculations of endophyte-free *T. cacao* leaves, with each of these microbial organisms individually, induce changes in the expression of hundreds of host genes. The affected genes in all cases include many genes with known defensive functions, suggesting a general "non-self" response. However, the endophyte treatments differ from the pathogens by greatly up-regulating the expression of defense genes that contribute to host resistance to *Oomycetes* pathogens such as *Phytophthora*. Furthermore, tissues from plants inoculated with *C. tropicale* exhibited increased lignin and cellulose content, reduced maximum rates of photosynthesis, and enrichment of nitrogen-15 and carbon-13 isotopes. Given the potential that host resistance to pathogen and herbivore species is influenced by the diversity and composition of the entire endophyte community, results from experiments to uncover the diversity and the function of the uncharacterized microbiome associated with cacao will be discussed.

Poster #133

Interactions between a cotton phytopathogen and the host using a genomics analysis

ENRIQUE MEDRANO, Whitney Mantooth, Kendall Woolf, Alois Bell

USDA-ARS, College Station, Texas USA

Cotton (*Gossypium hirsutum* L.) is an economic crop grown worldwide. Numerous *G. hirsutum* polyploids have been sequenced. Bacterial infections of cotton can cause major yield losses. *Pantoea ananatis* is a known bacterial pathogen of both cotton buds and bolls. Thus, we conducted a whole genome analysis of the infectious *P. ananatis* strain CFH 7-1. Strain CFH 7-1 is associated with transmission by the cotton fleahopper (*Pseudatomoscelis seriatus*). Using Roche technology, a high quality draft of the whole genome was generated for the purpose of finding virulence genes to match respective host avirulence genes from published cotton genomes. Both a chromosome (4.5 Mb) and extrachromosomal DNA (2.5 kb) were identified. Annotation of the bacterial genome (4405 CDS) revealed putative pathogenicity elements including type IV and VI secretion systems. Avirulence genes in the recently published allotetraploid *G. hirsutum* acc. TM-1 have been potentially identified. This work assists in developing techniques to minimize damages associated with *P. ananatis* infections by analyzing both the host and phytopathogen genomes.

Poster #134

Sorghum root exudation is affected by genotype and growth media: implications to the rhizosphere microbiome

SARAH MILLER, Courtney Jahn, Jay Kirkwood, Corey Broeckling, Jessica Prenni

Colorado State University, Fort Collins, CO USA

Rhizodeposition is the process by which plant roots release small molecules into the rhizosphere. This pool of small molecules, known as root exudates, contributes to plant health by facilitating nutrient acquisition from the soil, mediating plant-microbe interactions and protecting against pathogens. Root exudates fluctuate with changes in the environment, microbial activity, and can vary due to plant genetic background and developmental growth stage. Further, root exudates can change the composition of the soil microbial community; this has been proposed as a major mechanism by which plants sustain a unique microbiome. In model plant species, soil type is noted to influence the rhizosphere-associated microbial communities. In this study, we evaluated the effect of differing growth media on the rhizodeposition of *Sorghum bicolor*, a globally important and genetically diverse crop species. Above and below ground morphological, physiological and biochemical traits were measured in sorghum varieties grown in sand, soil, fritted clay and a hydroponic system. Non-targeted metabolomics was conducted using liquid chromatography (LC-) and gas chromatography (GC-) mass spectrometry (MS) to determine variation in root exudate composition and quantity among the four media-types. We detected significant variation among morpho-physiological trait classes including increased leaf area and fine root production in soil and hydroponic systems. Root exudate composition and abundance varied by sorghum genotype and by growth media. Future studies will evaluate the influence of genetic background and growth media on root exudation in response to drought stress.

Poster #135

Identification and mapping of Quantitative Resistance to Powdery mildew in Flowering dogwoods (*Cornus florida* L.)

Lipi Parikh, MARGARET MMBAGA

Tennessee State University, Nashville, Tennessee USA

Powdery mildew disease on *Cornus florida* is one of the most destructive diseases in nursery production throughout the Southeastern US since the early 1990's. Efforts to breed for resistance have been slow and only a few cultivars have been rated resistant. Quantitative trait Loci (QTL) approach was used to study the inheritance of powdery mildew resistance in Pseudo F₂ populations derived from controlled crosses between two phenotypically distinct parents, Cherokee Princess (Susceptible) and MI9 (Resistant selection). Disease severities recorded for parental clones and progeny populations in greenhouse environment showed continuous distribution of disease severity indicating quantitative resistance to powdery mildew. Identification of microsatellite markers that may be linked to resistance in the segregating progeny population was done; a total of 105 markers were selected from a previous *C. florida* genetic maps. Amplification of microsatellite regions within the genome of parent population was done and polymorphisms were identified. A total of 51 polymorphic markers at the distance of 10 cM were selected to identify polymorphisms in the progeny. The electrophoretic bands were then scored for construction of a genetic linkage maps. The markers identified for resistance/susceptibility can be used in marker assisted breeding programs for dogwoods and for generating future linkage maps with high saturation.

Poster #136

Induced systemic resistance mode of action by bacterial Biological control agents against powdery mildew in *Cornus florida*

Emily Rotich, MARGARET MMBAGA

Tennessee State University, Nashville, TN USA

Parama Sikdar

Washington State University Wanatchee Washington USA

Bacterial biological control agents (BCAs) protect plants against pathogens by various mechanisms and some promote plant growth. Three bacterial BCAs B17A (*Stenotrophomonas* sp.), B17B (*Serratia* sp.) and IMC8 (*Bacillus* sp.) that were previously shown to control powdery mildew in *Cornus florida* were evaluated for mechanism of action. The objectives of this research was to determine if the BCAs promote plant growth and induce systemic resistance (ISR) by inducing defense enzymes, and accumulation of pathogenesis related proteins. Vernalized seeds treated with the BCAs for 24h before planting were used to assess growth parameters; all BCAs promoted growth significantly with higher chlorophyll content, plant height and dry weight compared to non-treated seedling. Healthy leaves from non-treated plants were spray-inoculated with 24- h-old bacterial cultures of approximately 3×10^9 cfu/ml; changes in peroxidase (PO) and polyphenol oxidase (PPO) activities was measured at 480nm at 15, 26, 48 and 74 h time points after treatment. Total RNA from the leaves was extracted, reverse transcribed and used to amplify pathogenesis related genes using PR1, PR2 and PR5 primers. An increase in PR1 proteins, PO and PPO was observed on leaves treated with B17A only suggesting that only *Stenotrophomonas* sp. seems to have ISR as a mode of action, while *Serratia* sp, and *Bacillus* sp. have a different mode of action.

Poster #137

Molecular Phylogenetic Analyses of Fungal Diversity in Agarwood from *Aquilaria malacensis*

MUNADIAN MUSALAM, Tsuyoshi Yoshimura

Kyoto University, Kyoto Japan

Makoto Yoshida, Yuiji Nakada

Tokyo University and Agriculture Technology, Tokyo Japan

Agarwood, a dark resinous wood found in the trees of South East Asia such as *Aquilaria* sp., has been realized as a high valued non-timber forest product. Since agarwood is a biological product of defense reaction against fungal infection. Artificial formation of agarwood by forcible infection using specific fungus can be useful option for its industrial production. For this purpose, identification of the fungus responsible for agarwood formation must be needed, whereas the available information is limited. In the present study, we investigated the fungal community existing in agarwood of *Aquilaria* sp. by PCR-DGGE, and compared it with that in healthy wood for identification of the causal fungus for agarwood formation. Eight wounded *Aquilaria malacensis* Trees were selected from two different islands in Indonesia. Genomic DNAs were extracted from 90 mg of milled wood samples using DNeasy Plant Mini Kit. After the reaction, PCR products were analyzed by electrophoresis on 1% agarose gel, and then applied for DGGE analysis. The separated DNA fragments were applied for sequencing analysis. Total 124 of DNA bands were separated with the different mobility on the gel for samples from Kalimantan, for samples from Sumatra, 85 of DNA bands were separated.

Ascomycetes and Basidiomycetes were discovered in both area. PCR-DGGE obtained overall data of fungi species that existing in natural agarwood. Another studies reported that several fungi were isolated from agarwood. In the present study, we identified more fungal species, suggesting that agarwood might be formed by the association of multiple fungus.

Poster #138

Isolation of endophytes promoting plant growth and withanolides production from *Withania somnifera*

SHIV PANDEY, Alok Pandey, Sucheta Singh, Ramesh Kushwaha, Madhumita Srivastava, CS Vivek Babu, Madan Gupta, Alok Kalra

CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow India

Withania somnifera is a medicinally important plant and used for centuries in Ayurvedic medicines. It produces withanolides having pharmacological activities such as physiologic and metabolic restoration, nerve stimulant, anti-arthritis, anti-ageing, cognitive function, improvement in the elderly and recovery from neurodegenerative disorders. In the present work, we have used endophytes to promote plant growth and withanolides production in *W. somnifera*. Endophytes are the plant-associated fungi and bacteria that reside within tissues of their host plants without causing any disease symptoms or harm. Endophytes promote plant growth, protect plant from environmental stresses and are source of therapeutically important secondary metabolites. Occasionally, endophytes induce secondary metabolite production in host plant. Here, a total number of 44 endophytes isolated from leaves, roots and seeds of *W. somnifera* were characterized. Endophyte free one month old seedlings of *W. somnifera* were inoculated with individual isolated endophytes and grown in pots filled with autoclaved soil and vermicompost mixture. Primary plant productivity in terms of photosynthetic rate (Net CO₂ assimilation), stomatal conductance, transpiration rate, and biomass were measured and noticed that few endophytes inoculation significantly increased primary productivity of *Withania* plants. Furthermore, we have observed that few endophytes also enhanced withanolides production in leaves and roots of *W. somnifera* plant. This study found compelling evidences that *W. somnifera* has useful endophytes having potential in promoting plant growth and withanolides production.

Poster #139

Does long-term no-till shift microbial communities in dryland wheat cropping systems?

Chuntao Yin, Nicholas Mueth, Scot Hulbert

Washington State University, Pullman, WA USA

Dipak Sharma-Poudyal

Oregon Department of Agriculture, Salem, OR USA

Kurtis Schroeder

University of Idaho, Moscow, ID USA

Aaron Prescott

University of Tennessee, Knoxville, TN USA

TIMOTHY PAULITZ

USDA-ARS, Pullman, WA USA

No-till is becoming more prevalent in dryland wheat in the Pacific Northwest to manage soil erosion, but some diseases such as *Rhizoctonia* become more serious during the transition from conventional tillage. However, in long-term no-till fields, these diseases are not a problem. Are microbial communities associated with this suppression? We used pyrosequencing with 16S rDNA primers to compare the bacterial community in bulk and rhizosphere soil of wheat in two locations in ID and WA, comparing long-term no-till plots side-by-side with conventional tillage plots. We also looked at fungal communities at three locations using pyrosequencing with ITS primers. The position of the sample (rhizosphere vs bulk soil) had a much stronger influence on bacterial communities than tillage practices. Families Oxalobacteriaceae, Micrococcaceae, Sphingobacteriaceae, Flavobacteriaceae, Pseudomonadaceae, Microbacteriaceae, and Enterobacteriaceae were more abundant in rhizosphere than bulk soil. Acetobacteraceae, *Spartobacteria* genera incertae sedis, Solirubrobacteraceae, Methylobacteriaceae, Conexibacteraceae, and Lamiaceae were higher in bulk soil than in the rhizosphere. Only two families, Rhodospirillaceae and Solirubrobacteraceae, were higher in no-tilled compared to conventional tillage soil in both years. In contrast, Chitinophagaceae were higher in the conventionally tilled soil. Preliminary results of fungal community in bulk soil showed that Ascomycota made up over 70% of the sequences, and some of the most dominant genera were in the families Chaetomiaceae and Lasiosphaeriaceae, which are cellulose decomposers. *Phomopsis* and *Trichocladium* were more dominant in no-till soils in all three locations. *Ulocladium* was more dominant in conventionally tilled soils. Fungi may be more strongly influenced by tillage practices than bacteria, especially rhizobacteria.

Poster #140

Deciphering Soil and Plant Microbiomes Associated with Suppression of Soybean Diseases

HALEY PFAFFE, Shinyi Marzano, Darin Eastburn

University of Illinois, Urbana, IL USA

Leslie Domier, Adam Davis

USDA-ARS, Urbana, IL USA

General disease suppression of soilborne pathogens can add to the toolbox of strategies for disease management. However, there has been no reliable predictor for such phenomena. This study was undertaken to identify the microbiome indicators for soil suppressiveness to sudden death syndrome (SDS) of soybeans. Five cover crop treatments (rye, vetch, mustard, rye+vetch, fallow) and two tillage methods (chisel plow, ridge till) were compared. Each cover crop plot was divided to receive both tillage treatments, and there were four replications of this experiment. Soybean plants were evaluated for naturally occurring foliar and root diseases at the R6-R7 growth stage. Root, rhizospheric soil, and bulk soil samples were taken early in the season, and DNA was extracted for PCR amplification, targeting 16S bacteria and archaeal, ITS, ammonia monooxygenase, nitrous oxide reductase, and ammonia generating nitrite reductase genes, for subsequent high throughput sequencing. Our results have shown no significant differences in microbial populations of the cover crop treatments. However, there are several notable differences with the tillage treatments. Chisel plowed plots had increased levels of the genera, *Verrucomicrobia*, *Glomeromycota*, *Proteobacteria*, and *Actinobacteria*. Ridge tilled plots show increased levels of *Streptophyta*, *Firmicutes*, and *Crenarchaeota*. These differences show that there are several factors that play important roles in shaping the structure of microbial disease suppressive communities as related to SDS. Our ongoing study demonstrates that it is possible to delineate microbiome profiles that correspond to general disease suppression, and highlights the importance of plant health with respect to field treatments.

Poster #141

Rhizobiome Responses to New Tomato Rootstock Systems

RAVIN POUDEL, A Jumpponen, M Kennelly, C Rivard

Kansas State University, Manhattan, KS USA

Karen Garrett

University of Florida, Gainesville, FL USA

Microbes in the rhizosphere are critical for nutrient exchange between plants and the soil, determining plant performance and health. These microbes are under constant biotic and abiotic pressure, and fluctuate with external interventions such as agricultural practices. We evaluated the impacts of grafting and rootstock genotypes on bacterial communities residing in the roots and rhizosphere of tomatoes, by using high-throughput sequencing and community analysis. Our study included a non-graft (cultivar BHN589), a self-graft (BHN589), and two hybrid grafts: (BHN 589/ BHN RT1028, BHN589/RST-04-106). 16S rRNA sequences revealed strong evidence ($p < 0.001$) for more diverse bacterial communities in the rhizosphere compared to the root samples in all the treatments, while the majority (85%) of identified (Operational Taxonomic Unit) OTUs were shared. Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Planctomycetes were the most abundant phyla in all the rootstock-scion combinations, where the proportion of the last three phyla decreased in the roots. The number of unique OTUs in the rhizosphere was twenty times higher than in roots. OTUs specific to rootstock genotypes, and to grafted versus non-grafted plants, were identified for both the root and the rhizosphere, and a substantial increase in the number of unique OTUs was revealed in grafted plants. The results of this study provide insights into the roles of grafting and rootstock genotype in the selection of microbial taxa. Understanding the mechanism of microbial selection by the plant genotype and grafting will ultimately support improved vegetable production with grafting techniques, thereby supporting farming practices.

Poster #142

Phytobiome networks: A framework for evaluating resilience and controllability

RAVIN POUDEL, Ari Jumpponen

Kansas State University, Manhattan, KS USA

Karen Garrett

University of Florida, Gainesville, FL USA

Network analyses of phytobiomes provide a new means for visualization of biological interactions among plants and microbial communities at the system level. Network science tools permit integration of information about communities and across 'omics levels. New network perspectives have the potential to inform crop improvement strategies based on phytobiomes. We present a framework to illustrate the utility of network analysis and use of null models, drawing on data from several phytobiome projects, along with exploration of parametric and nonparametric statistical methods. We also highlight inherent challenges associated with phytobiome data and phytobiome network interpretation, such as compositional bias, spurious correlations, and unequal sequencing and sampling depth. Our primary analyses were based on the relative frequency count data of DNA sequences, where a network node represents a taxon and an edge exists between two taxa if their frequency is correlated or proportional across samples. Network topologies can be summarized thorough node degree distribution, diameter, modularity, motif size and distribution, and connectance – metrics that allow for comparisons of networks from different treatments. Such comparisons provide further insights for evaluating plant-microbe systems and defining the concept of system resilience and controllability in the phytobiome. Cautious interpretation of network parameters may be designed to contribute to the general understanding of plant-microbe interactions, and thus to support design of robust farming and crop health management practices.

Poster #143

Bulk soil community structure and its impact on rhizosphere microbiomes of *Arabidopsis thaliana* defense signaling mutants and transgenic lines overexpressing antimicrobial defensins.

BHARATH PRITHIVIRAJ, Yingqing Guo, Myoung-Hwan Chi, Kelly Craven, Nick Krom

The Samuel Roberts Noble Foundation, Ardmore, OK USA

Dilip Shah

Donald Danforth Plant Science Center, St Louis, MO USA

Plants are known to exert selective pressure on soils to specifically shape and restructure microbial communities in the rhizosphere. Addressing our understanding of “source” to “sink” exchanges between rhizosphere microbes and plants could potentially enhance sustainability practices for the development of crop varieties that optimize beneficial functions. Further, while root exudation has been shown to have a major influence on rhizosphere microbiome composition, virtually nothing is known about the role of plant defense signaling genes and antimicrobial compounds in shaping these communities. Soils associated with canola (family Brassicaceae) farms from Western Oklahoma were sampled and characterized for parameters like pH, organic matter, P-N-K composition and soil vitality. *A. thaliana* wild type plants were grown alongside salicylic acid (*npr-1*) and jasmonic acid (*jar-1*) signaling mutants, and compared with lines overexpressing a *Medicago truncatula* antifungal defensin gene (Kaur et al., 2012) in these bulk soils. MiSeq 16S rDNA libraries were generated using 515F and 806R primers specific to the V4 hypervariable region for bulk soils, rhizosphere and rhizoplane regions to infer community composition shifts between these microhabitats. Analysis of sequence data reveals dominant phyla such as *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Planctomycetes*. Bioinformatic analysis was performed using UPARSE (Edgar et al. 2013) to remove chimeric sequences and pick OTUs. Further analysis using QIIME revealed that soil pH influenced the community assemblages in bulk soils. PICRUST analysis reveals an enrichment of niche specific roles like carbon fixation, nitrogen metabolism and ABC transporter pathways in these soil bacterial taxa.

Poster #144

Integrated Biorational Management of Leaf and Stripe Rust of Wheat

MUHAMMAD MOHSIN RAZA, Muhammad Aslam Khan, Muhammad Yaseen

University of Agriculture Faisalabad, Faisalabad, Punjab Pakistan

Wheat rusts encompassing leaf and stripe rust are most important diseases of wheat worldwide, which engendered to hefty wheat yield losses each year. A biorational approach using plant extracts, antagonists and bioproducts is required for sustainable wheat leaf and stripe rust management. A total of nine treatments including fungicides and the plant extract (*Azadirachta indica*, *Datura stramonium*, *Nicotiana tabacum* and *Ricinus communis*); the biological control agents (*Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*); the bio products (Bio Magic and Unigrow); and two conventional fungicides (Folicur® and Tilt®) were examined during the 2013-14 growing season for their ability to manage leaf and stripe rust epidemics, on moderately susceptible cultivars ‘Sehar-06 and Shafaq-06’ respectively. Assessments consisting of average coefficient of infection (ACI) of disease were made at seven day intervals during the course of the epidemic and the data were analyzed using linear mixed model analysis under repeated measures design. All the treatments reduced average coefficient of infection to a greater extent as compared to control. In case of leaf rust, biorationals including *A. indica* (25.92%), *T. harzianum* (28.83%), Bio Magic (24.92%) and Folicur® (17.17%) revealed minimum leaf rust ACI as compared to control (58.33%) while managing stripe rust, *B. subtilis* (44.58%), *R. communis*

(38.41%), Bio-Magic (36.66%) and Folicur® (30.00%) demonstrated minimum stripe rust ACI. Since these biorationals consistently reduced average coefficient of infection, hence they may be candidates for use in integrated programs with conventional fungicides.

Poster #145

High Throughput Soil Printing: A micro-scale tool to isolate and study rhizosphere species

BRADLEY RINGEISEN, Lisa Fitzgerald, Preston Fulmer

United States Naval Research Laboratory, Washington, DC USA

Karina Rincon

Florida International University, Miami, FL USA

Peter Wu

Southern Oregon University, Ashland, OR USA

We have demonstrated a high throughput soil printing method that enables the study and culture of the rhizosphere at the microscale. Traditional high throughput methods for isolating microorganisms from soil require pre-processing steps that remove the living species from their solid-phase microniche, creating a liquid-phase sample. This pre-processing destroys near-neighbor relationships that could be crucial to culturing microorganisms and understanding their interactions with plants. We will present experiments that demonstrate high throughput isolation of soil micro-particles while maintaining microorganism viability and their spatial proximity to one another at a 100 micrometer scale. The approach is based on a modified laser induced forward transfer technique termed biological laser printing, or BioLP. Because BioLP uses a forward transfer mechanism from a flat printhead rather than extrusion through an orifice or capillary, soil can be printed directly from the solid-phase with no clogging, rather than from liquid slurry. Tunable amounts of soil were printed at rates exceeding 20 micro-particles per second. Microbial viability, culturability and significant morphological diversity were demonstrated post-soil printing. Both pure microbial cultures (isolates) and mixed consortia cultures were observed to be isolated in a single step. We hope to use this technology to rapidly isolate the specific microbial communities that populate the root/soil interface while maintaining the spatial proximity and naturally evolved interactions between the different species present in this complex microenvironment.

Poster #146

Unlocking the potential of disease control within the phytobiome: A case study exploring the boxwood ectorrhizosphere

YAZMIN RIVERA, Jo Anne Crouch

USDA-ARS, Beltsville, Maryland USA

Precious Ndukwe

USDA-ARS, Charles Herbert Flowers High School, Springdale, Maryland USA

Lily Cornelius

USDA-ARS, Eleanor Roosevelt High School, Greenbelt, Maryland USA

Lisa Beirn

Rutgers University, New Brunswick, NJ USA

There are numerous biocontrol products available in the commercial market to control plant diseases. This practice is widely adopted and has yielded many notable successes, helping plant health professionals to reduce or eliminate chemical fungicide usage. However, biocontrol is not always effective. This unpredictability may be due to the complexities of individual phytobiomes and the interactions that take place between the living biological control agents and with the native microbiota. In this study, we explore the fungal component of the boxwood rhizosphere to identify resident natural enemies of the newly emergent boxwood blight pathogen, *Calonectria pseudonaviculata* (*Cps*). Rhizosphere soil from 40 mature boxwood plants from two arboreta collections was sampled (20 species/cultivars) and metagenomic analyses performed from 454 pyrosequencing of the ITS region. *Fusarium* and *Mortierella* were the most widespread and dominant fungal genera. Thirty species of *Trichoderma* – a genus known for its antagonistic properties – were identified, but were minority constituents in all but one sample. *Trichoderma* cultured from the soil samples reduced *Cps* growth by as much as 99.4% in dual-culture experiments, outperforming commercial strain T22. Growth inhibition varied among the different *Trichoderma* isolates within species, and isolates of *Cps* responded differently to individual *Trichoderma* isolates, suggesting a genotype x genotype interaction. These data show that indigenous fungal communities in the native phytobiome may be powerful weapons for disease control.

Poster #147

Bacterial community dynamics in industrial algae production systems

ADAM ROBBINS-PIANKA

University of Colorado, Boulder, CO USA

Scott Fulbright, Donna Berg-Lyons, Stephen Chisholm, Rob Knight, Kenneth Reardon

Colorado State University, Fort Collins, CO USA

Microalgae are a promising feedstock for biofuel production with the potential to make a large impact on displacing fossil fuels. However, a major challenge is consistent and robust algae growth during the scale-up process in bioreactor systems. The scale-up process consists of elite algae strains such as *Nannochloropsis salina*, which start in small 5 mL culture and scale to dozens of 200 L cultures. Large cultures are often comprised of complex communities of bacteria and are difficult to manage as specific bacteria can promote or reduce algae growth. For successful algae cultivation, we must improve our understanding of ecology in algae production systems. In this study, we characterized bacterial community richness, structure, and composition within industrial algae bioreactors during the scale-up process, through time, and during different algae growth rates. We analyzed 16S rRNA amplicon sequence data and determined that bacterial communities in small, medium and large cultures were significantly different. Large systems contained richer bacterial communities compared to small cultures based on phylogenetic distance and OTU count. Additionally, a single bacterium within the Saprospirae class was found in 100% of samples, with an average relative abundance of $34.7\% \pm 14.6\%$. Also, we sampled poorly performing large cultures, which a Deltaproteobacteria (spirobactillales) was observed at high relative abundances. This is the first study to characterize bacterial communities during the scale-up process, setting a foundation for crop protection studies in algae systems.

Poster #148

Composition of Fungal Communities in Soil and Endophytic in Raspberry Production Systems

EMILIANA ROMAGNOLI, Rodrigo Mendes

EMBRAPA Environment, Jaguariuna, Sao Paulo Brazil

Christopher Dunlap

USDA, National Center for Agricultural Utilization Research, De Peoria, IL USA

Fungi play important roles as decomposers, plant symbionts and pathogens in soil. While endophytes are microorganisms that dwell within plant tissues and have a symbiotic association with the host. The structures of fungal communities in the soil and in endophytic association are dependent up complex interactions with the environment and the host. These two communities have a great influence on plant health and development. Using culture-independent fungal community profiling, we investigated the effects of fertilizer (composted dairy solids + mustard seed meal) on fungal communities in soil and endophytic in a raspberry production system. During the study we evaluated the impact of primer selection ITS1 vs ITS2. We characterized the communities for both spring and fall time periods. The results show that the soil communities are dominated by Ascomycota, and Basidiomycota in soil, while the endophytes were primarily Ascomycota. The relative abundances of certain taxa, such as Capnodiiales, were more predominant in composted soil (8%) than the control (4%). There were no significant differences identified in the endophytic communities between the two treatments. Further research should elucidate the specific roles of these fungal taxa in raspberry soils and endophyte, and on the heath of the plant. To advance the ecological management of crop soils, understanding is needed of how beneficial microbial relationships can be fostered in these production systems.

Poster #149

Population genetics from the obligate biotrophic component of phytobiomes: Challenges and potential solutions

CATALINA SALGADO-SALAZAR, Yazmín Rivera, Martha Malapi-Wight, Daniel Veltri, JoAnne Crouch

USDA-ARS, Beltsville, MD USA

Recent advances in molecular biology have opened a world of possibilities for non-model plant pathogen research. In particular, the phytobiome-restricted lifestyle of downy mildews and other obligate plant pathogens presents several unique challenges that are poorly addressed using traditional experimental approaches, but are well resolved through next generation technologies. Conventional genotyping techniques may be unable to account for the presence of multiple genotypes, allelic variation, or minor alleles within a community. In contrast, targeted genotyping-by-sequencing approaches allow us to study both global pathogen populations and community structure at the local level (individual plant, different tissues, etc.), since hundreds of markers can be employed simultaneously and at high depth of coverage. Our research of the newly emergent oomycete pathogen responsible for impatiens downy mildew (IDM) disease, *Plasmopara obducens*, is providing a striking example of how obligate community structure may be underestimated. Using standard fragment analysis of 37 polymorphic SSR markers developed from the *P. obducens* genome, six populations were identified, and clearly differentiated between pre- and post-epidemic populations. Illumina sequencing of these markers (~20k x) showed in some instances, SSR flanking regions were highly variable. For example, at one bi-allelic SSR locus evaluated for four IDM samples, GBS data identified 32-46 variants and three alleles. SSR genotyping grouped these samples as a

single genotype, but GBS data showed each as unique, and likely populated by a community of *P. obducens*. Similarly, droplet digital PCR experiments observed allelic ratios departing from 1:1, with several samples exhibiting 3:1 ratios.

Poster #150

Plant-secreted LysM proteins may play a role in defense suppression for maintaining symbiosis during arbuscular mycorrhiza

ALEXA SCHMITZ

Cornell University, Ithaca, NY USA

Maria Harrison

Boyce Thompson Institute for Plant Research, Ithaca, NY USA

Arbuscular mycorrhizal fungi are obligate biotrophs, providing their host plant with mineral nutrients in return for carbon. Throughout the relationship, the fungus maintains an intracellular lifestyle in the plant roots. Typically, plants respond to the presence of microbes through recognition of molecular patterns, which induce pattern-triggered immunity (PTI). Chitin is an elicitor of PTI and can be found in cell walls of all fungi. How AM fungi are able to avoid this defense is still unknown; however, lessons may be learned from plant-pathogen effectors that bind and sequester chitin through their lysin motif (LysM) domains to inhibit PTI. The LysM domain binds chitin and related molecules, and can be found in at least five different plant protein families, one of which consists of a small, secreted protein with a single LysM domain. These plant-secreted LysM (SLM) proteins may mask fungal chitin from the plant's own receptors during symbiosis. We have identified three *Medicago truncatula* SLM genes expressed only during AM symbiosis. SLM promoter-GUS fusions reveal that these SLM genes are expressed in cells containing arbuscules, the fungal structure where nutrient exchange takes place during the symbiosis. Furthermore, using fluorescent protein fusions, we have shown SLM proteins localize around arbuscules. Initial experiments suggest that ectopic expression of AM-specific SLM genes results in greater AM fungal colonization, leading us to investigate if SLM proteins inhibit chitin-elicited ROS production. We are currently testing if SLM proteins bind chitin. Our work attempts to uncover a mechanism for suppression of defense during AM symbiosis.

Poster #151

Fungal diversity as a component of tropical phytobiomes: a focus on *Hevea* and *Micrandra*

DEMETRA SKALTSAS, Priscila Chaverri

University of Maryland, College Park, Maryland USA

Lisa Castlebury

USDA-ARS Systematic Mycology & Microbiology Lab, Beltsville, MD USA

Hevea brasiliensis is the primary species for tapped natural rubber and plantation grown trees suffer from a variety of diseases that reduce production or destroy entire plantation areas. Endophytes obtained from the wild that have a highly specialized relationship with their hosts are considered ideal candidates for developing biological controls against diseases of that host. The objectives of this project were to (1) characterize and compare the diversity of culturable and unculturable endophytes obtained from wild *Hevea* spp. and *Micrandra* spp. (Euphorbiaceae), and (2) explore host preference or host recurrence across selected species from *Hevea* and its sister genus, *Micrandra*. Plant tissue samples were collected from adult and seedling trees located in the Amazon basin (Peru and Brazil). DNA was extracted from adult sapwood tissue and ITS2 was amplified. Sequencing was performed on an Illumina MiSeq. Adult sapwood tissue and seedling twigs were also plated onto PDA*. Isolated fungal endophytes were identified using ITS nuclear ribosomal DNA. In the adult trees, Hypocreales (Ascomycota) were by far the most abundant group using both methods. *Trichoderma* spp., a well-known mycoparasite, was the most abundant using culturing techniques and *Acremonium* cf. *strictum* using metagenomics. In seedlings, Diaporthales were the most abundant group. Interestingly, no *Trichoderma* species were isolated from the seedlings. Understanding the fungal diversity, host associations or preferences, and spatial distribution of *Hevea* and related endophytes may provide additional tools for an integrated approach to disease management of plantation grown trees.

Poster #152

Soil chemistry drives microbial community structure and nitrogenase gene abundance in the rhizospheres of *Miscanthus x giganteus* and *Panicum virgatum*

CHINMAY SOMAN, Dongfang Li, Angela Kent

University of Illinois, Urbana, IL USA

Daniel Keymer

University of Wisconsin, Stevens Point, WI USA

Miscanthus x giganteus and *Panicum virgatum* are promising bioenergy feedstock crops suitable for the temperate zone. While we have previously shown that *Miscanthus* benefits from nitrogen fixation by associative diazotrophs, the effects of plant species and soil chemistry on community structure and nitrogen fixation potential of microbiomes associated with these plants are currently not well understood. We characterized rhizosphere and endophytic microbial communities of both crops from eight research sites in Illinois with a range of soil chemistry parameters. Microbial and diazotrophic communities were assessed in the rhizosphere and

endophytic compartment using fragment length analysis of the ribosomal intergenic spacer and the *nifH* gene respectively. *nifH* gene abundance in the rhizosphere was measured with quantitative polymerase chain reaction. Microbial and diazotrophic taxa in the *Miscanthus* rhizosphere at each site were identified by sequencing the 16S rRNA gene and *nifH* gene respectively. Multivariate statistical analyses of these data show that while bacterial and diazotrophic endophytic communities differed substantially between plant species, site-to-site variation predominated differences between rhizosphere communities. *nifH* abundance in the rhizosphere was positively correlated with abundance of ammonia and bioavailability of iron in the soil but negatively correlated with abundance of other soil nutrients including total nitrogen and nitrates. Indicator species analysis revealed enrichment of Proteobacteria, specifically Deltaproteobacteria, in sites with greater *nifH* abundance. This study demonstrates that associative diazotrophs could contribute to the sustainability of bioenergy feedstock production by enhancing nitrogen availability in low-fertility soils, especially with improved iron bioavailability *via* site selection and/or soil amendments.

Poster #153

***Ralstonia solanacearum* lipopeptide induces chlamydospore formation followed by bacterial entry in close encounters with fungi**

JOSEPH SPRAKER, Nancy Keller

University of Wisconsin, Madison, WI USA

Laura Sanchez, Pieter Dorrestien

University of California, La Jolla CA USA

The polymicrobial consortium within the rhizosphere communicates by chemical signaling that, ultimately, impacts survival in symbioses. Here we characterize the endosymbiotic interaction of two economically important plant pathogens, *Aspergillus flavus* and *Ralstonia solanacearum* mediated by a novel bacterial lipopeptide. Using a variety of histological techniques we show that fungal chlamydospore-like structures (CLSs) form in response to a diffusible compound produced by *R. solanacearum*. Phylogenetic analysis of the 16S ribosomal subunit indicates that *R. solanacearum* is nested amongst other described endofungal species. Imaging Mass-Spec (IMS) and targeted genetic deletion show this metabolite to be a new lipopeptide, here named ralsolamycin. Confocal scanning laser microscopy with a GFP producing *R. solanacearum* isolate confirms bacterial internal colonization of chlamydospores, indicating a newly described endofungal lifestyle for this important plant pathogen. Bioassays of related fungal species show that the CLS response to ralsolamycin is not conserved across all clades of *Aspergillus*, suggesting that there is some specificity to the perception of this compound.

Poster #154

Sequence populations of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in Maize lethal necrosis-affected areas of Uganda and Kenya

LUCY R. STEWART, Margaret G. Redinbaugh

USDA-ARS Corn, Soybean and Wheat Quality Research Unit, Wooster, Ohio USA

Saranga Wijeratne

Molecular and Cellular Imaging Center, Ohio State University-OARDC, Wooster, Ohio USA

George Mahuku

International Center for Tropical Agriculture (CIAT), CIMMYT, Nairobi Kenya

Andrew Kiggundu, Godfrey Asea

National Agricultural Research Organization, Entebbe Uganda

Anne Wangai

Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi Kenya

Charles Niblett

Venganza, Inc., St. Augustine, FL USA

Maize lethal necrosis is a devastating emerging virus disease in East Africa caused by dual infection with *Maize chlorotic mottle virus* (MCMV) and any virus in the family *Potyviridae*, such as *Sugarcane mosaic virus* (SCMV). Maize-infecting potyviruses are diverse and found worldwide. Over 40 maize samples collected across Uganda and Kenya in areas where MLN is severe were analyzed for virus populations by RNA deep sequencing and an in-house bioinformatics pipeline for virus identification. Collected SCMV sequences grouped into four clusters with over 10% nucleotide sequence divergence in the polyprotein open reading frame. SCMV coat protein coding sequences from these isolates clustered into three groups with similar nucleotide sequence divergence. In contrast, African MCMV sequences were distinguishable from US isolates but showed less than 1% nucleotide sequence variation in the coding and intergenic regions. Understanding virus populations causing MLN are likely to improve detection and containment methods, especially given the diversity of SCMV potyvirus populations that are likely contributing to disease.

Poster #155

Manipulating fungal microbiomes for plant stress resistance and improved yields in cultivated cotton

GREGORY SWORD

Texas A&M University, College Station, Texas USA

Beneficial fungal endophytes can confer protection to plants from a variety of stressors and improve yields in major agricultural crops. We have been systematically evaluating the ecological, physiological and agronomic effects of fungal endophytes originally isolated from cultivated cotton (*Gossypium hirsutum*). Using simple seed treatment protocols, individual cotton plants can be inoculated with endophytic fungi with resulting phenotypic effects detectable across the entire growing season. Through a combination of greenhouse assays and major field trials, we have demonstrated that the targeted manipulation of fungal endophytes in cotton can mediate resistance to multiple stressors including insects, nematodes and drought, with significant positive impacts on plant performance and yields in the field.

Poster #156

Deciphering plant and associated microbes genomic interactions

CECILIA TAMBORINDEGUY, Ordomb Huot, Freddy Ibanez, Julien Levy

Texas A&M University, College Station, TX USA

The importance of plant-associated microbes in plant fitness is widely recognized. These effects can result from the direct effect of the microbes on the plant, or indirectly by their effects on other plant-associated organisms such as herbivore insects or other pathogenic or beneficial microbes. Until recently, the identification of these plant-associated microbes, the sequencing of their genome and the deciphering of the molecular interactions with the host plant were limited by the fastidious nature of many of those microbes. The advancement of next-generation sequencing techniques has allowed overcoming these difficulties and has opened the door to the analysis of plant and its associated microbes interaction. Using RNAseq approaches we have obtained the transcriptome of a vector-borne plant pathogenic bacterium, '*Candidatus Liberibacter solanacearum*', in association with its vector, and we have evaluated the effects of this bacterium on the eukaryotic host as well as in other vector-associated bacteria. We propose to use the same approach to evaluate the effects of this microbe on the host plant and on other plant-associated microbes. Interestingly, this study will improve our understanding on the fastidious nature of this pathogen, and the mechanisms used to adapt to different insect and phloem environments where the bacterium thrives, including the strategies used to counteract immune defenses.

Poster #157

Bacterial cyanogenesis and its role in biocontrol of plant-parasitic nematodes

CHRISTOPHER TAYLOR

The Ohio State University, Wooster, OH USA

Plant-parasitic nematodes are among the most destructive plant pests, causing substantial economic losses to agronomic crops worldwide. Current methods of using bacteria as biocontrol agents for plant-parasitic nematodes have met with limited success in part due to limited knowledge about mechanisms of biocontrol and biotic factors that are important to rhizosphere persistence. Using a nematode bioassay we have screened over 10,000 bacterial isolates from a variety of natural sources (water, soil, roots) and identified over 50 different isolates of *Pseudomonas* that interfere with nematode growth and development. Over half of these strains also exhibited activity in plate and soil assays against other plant-pathogenic fungi, oomycetes and bacteria. Genome sequence analysis of all 50+ strains reveal the presence of many genes that are potentially involved in biocontrol activity including the production of antibiotics, siderophores, hydrogen cyanide (HCN), polysaccharides, and exoproteases. In several *Pseudomonas* strains we used both random and targeted mutagenesis to identify non-nematode lethal mutants. Testing of the non-lethal isolates for HCN showed significant reduction in HCN production. Loss of HCN production was correlated with reduced capacity to protect plants from plant-parasitic nematodes. Our data indicate that HCN is potentially an important compound produced by pseudomonads within the rhizosphere with activity against plant-parasitic nematodes.

Poster #158

The wheat phytobiome across four land management strategies

FRANCES TRAIL, Kristi MacCready

Michigan State University, East Lansing, Michigan USA

For many years, research has indicated that microbes can influence crop health and productivity. Current genomics methods provide the opportunity to comprehensively study intact microbial communities associated with a particular niche or treatment. Several studies have been conducted to investigate the differences in rhizosphere microbiota between crops or land management strategies, but less information is known about the microbiota inhabiting the plants. The wheat fungal and bacterial phytobiome composition was assessed at the Kellogg Biological Station Long-Term Ecological Research site, maintained for more than 25 years in a wheat/maize/soybean crop rotation under four management strategies (conventional, no-till, low nitrogen input, organic).

Illumina sequencing was performed to characterize the phytobiomes of three plant organs (leaf, stem, root) across three growth stages (vegetative, flowering, senescence) under the four management strategies. This study will begin to unravel the effects of land management strategies on crop phytobiomes, and determine the relationships among phytobiomes in different plant organs. Our ongoing study will also compare our results in wheat to the phytobiomes of maize and soy grown on the same sites.

Poster #159

Interaction between persistent viruses of common bean and pepper and four acute viruses

Surasak Khankhum, Cesar Escalante, RODRIGO VALVERDE

Louisiana State University Agricultural Center, Baton Rouge, LA USA

Plant endornaviruses are persistent RNA viruses that do not cause detectable symptoms. In contrast, acute viruses cause symptoms and in most cases disease. Many common bean (*Phaseolus vulgaris*) genotypes are single or double infected with *Phaseolus vulgaris* endornavirus 1 and *Phaseolus vulgaris* endornavirus 2. Similarly, bell pepper (*Capsicum annuum*) and other capsicum genotypes are infected with Bell pepper endornavirus. To study potential interactions between endornaviruses and acute viruses, we selected two lines of the bean cultivar Black Turtle Soup (BTS) and two lines of bell pepper Marengo (one endornavirus-infected line and the other endornavirus-free) and four plant viruses, *Pepper mild mottle virus* (PMMoV), *Tobacco ringspot virus* (TRSV), *Tomato spotted wilt virus* (TSWV) and *Sun hemp mosaic virus* (SHMV). In the case of the endornavirus-infected BTS, mechanical inoculations with TRSV resulted in chlorotic lesions, systemic necrosis, and stunting; in contrast, the endornavirus-free BTS line reacted with necrotic local lesions without systemic infection. Inoculation with SHMV resulted in similar systemic mosaic symptoms in both lines. Virus and viral RNA titers of SHMV were evaluated by ELISA and qPCR respectively. Although ELISA values varied between the two BTS lines, qPCR did not show significant differences. The two Marengo pepper lines were mechanically inoculated (individually) with PMMoV and TSWV. Symptoms were recorded, and acute virus titers were determined by ELISA. The overall results did not reveal differences between the two bell pepper lines. Data obtained in this investigation suggest that limited interactions take place between endornaviruses and acute viruses.

Poster #160

Effects of dietary supplements on the rat fecal microbiome and metabolome: a paradigm for phytobiome research

ARCHANA VASANTHAKUMAR, Ramon Molina, Joseph Brain

Harvard School of Public Health, Boston, MA USA

Ralph Mitchell

Harvard University, Cambridge, MA USA

Exposure biology in animals entails studying the interactions of exogenous factors such as diet and environment with endogenous factors such as the microbiome and metabolome. Similarly, in plants, exogenous factors such as soil and fertilizers interact with endogenous factors such as their microbiome and metabolome. Therefore, we propose an exposure biology-based model for phytobiome research. Dietary modifications can cause dramatic changes in the microbiome that alter the host's physiological state. Recent studies suggest that intake of prebiotics (foods that increase beneficial microorganisms in the intestinal tract) might affect health status. We hypothesized that dietary supplementation with a health drink Pairogen®, which contains fruit vinegars, sweeteners and minerals, could alter the rat fecal metabolome and microbiome. Ten pregnant rat dams were assigned to either tap water or 1% (v/v) Pairogen-supplemented drinking water from the start of pregnancy until the end of lactation. Weaned pups were provided with the same assigned drinking water until age 42 days. Two rat pups from each pregnant rat (n =10 rats/treatment group) were followed. At 21 and 42 days, fecal samples were analyzed using untargeted metabolomics and pyrosequencing of the 16S rRNA gene. Microbiome data indicate a shift in microbial community composition and structure. Fecal metabolite profiles indicate that Pairogen supplementation may contribute to increased glycogen metabolism, thereby impacting overall energy metabolism. We propose that the mammalian model presented here is a useful paradigm for phytobiome research. Specifically, we recommend characterization of the plant's microbiome and metabolome in response to various exposures during its life cycle.

Poster #161

Simple Synteny: An accessible tool for genome comparison

DANIEL VELTRI

USDA-ARS Systematic Mycology & Microbiology Lab and George Mason University, Beltsville, MD USA

Martha Malapi-Wight, Jo Anne Crouch

USDA-ARS Systematic Mycology & Microbiology Lab, Beltsville, MD USA

Understanding the co-localization of genetic loci amongst species is a frequent task in phytobiome research. While there are a number of programs currently available for generating syntenic maps, many are inaccessible to biologists who lack the required computer or scripting skills needed for proper installation or data preprocessing. We present here Simple Synteny, a free and open-source image generator for use in syntenic analysis. Simple Synteny provides an easy interface for comparing multiple genomes by only requiring the user to submit FASTA files for the genomes and genes of interest. The program employs gene color coordination

across genomes, indicator arrows for changes in gene direction and automatic contig and gene resizing to produce publication-quality images which are clear and simple to interpret. Directions for using the tool and example analysis is provided.

Poster #162

Improving turfgrass sustainability through a better understanding of the phytobiome

RUCHI VERMA, Scott Warnke, Dilip Lakshman, Geoffrey Rinehart

USNA/USDA-ARS, Beltsville, Maryland USA

Kevin Morris

NTEP/USDA-ARS, Beltsville, Maryland USA

Turf managers are being asked to reduce water, fertilizer and pesticide inputs on turfgrasses while environmental stress factors such as drought and heat are enhancing the need for these inputs to maintain turf health. One way to address this problem is to develop a better understanding of how turfgrass plants interact with microbial populations and the importance of these interactions for overall turfgrass health. Strategies are needed for the design of such experiments; For example, what strategies should be followed for sample collection, management of sample data, data analysis, and interpretation of the results. The objectives of this study are (1) to use metagenomic and other 'omics' studies to help develop an understanding the complex interactions between different microbial communities and their relationship with the host and its environment; (2) to design new strategies, protocols and pipelines which will help us to improve our understanding of the phytobiome of turfgrasses; (3) to use this information for designing robust experiments and protocols for sustainable turfgrass management. Additionally, bioprospecting strategies will be studied to determine if new products can be identified that will enhance the benefits of turfgrass for consumers and the environment.

Poster #163

Amplicon-based metagenomics identified candidate organisms in soils that caused yield decline in strawberry

XIANGMING XU, Thomas Passey, Fei Wei, Richard Harrison

East Malling Research, West Malling, United Kingdom

A phenomenon of yield decline due to weak plant growth in strawberry was recently observed in non-chemo-fumigated soils, which was not associated with the soil fungal pathogen *Verticillium dahliae*. Amplicon-based metagenomics was used to profile soil microbiota in order to identify microbial organisms that may have caused the yield decline. More than 2000 fungal or bacterial operational taxonomy units (OTUs) were found in these samples. Relative abundance of individual OTUs was statistically compared for differences between samples from sites with or without yield decline. A total of 721 individual comparisons were statistically significant. Based on further selection criteria, we focussed on 34 bacterial and 17 fungal OTUs and found that yield decline resulted probably from one or more of the following four factors: (1) low abundance of *Bacillus* and *Pseudomonas* populations, which are well known for their ability of suppressing pathogen development and/or promoting plant growth; (2) lack of nematophagous fungus (*Paecilomyces* species); (3) high level of two non-specific fungal root rot pathogens; and (4) wet soil conditions. This study demonstrated the usefulness of an amplicon-based metagenomics approach to profile soil microbiota and to detect differential abundance in microbes.

Poster #164

pltM is important in the phloroglucinol-mediated crosstalk between biosynthetic gene clusters for the antibiotics 2,4-diacetylphloroglucinol and pyoluteorin in *Pseudomonas protegens* Pf-5

QING YAN, Jeff Chang, Joyce Loper

Oregon State University, Corvallis, OR USA

Jennifer Clifford

USDA-ARS, Corvallis, OR USA

Pseudomonas protegens strain Pf-5 is a well-characterized rhizosphere bacterium known for its production of a diverse spectrum of antimicrobial secondary metabolites. The production of two of these metabolites, 2,4-diacetylphloroglucinol (2,4-DAPG) and pyoluteorin, is coordinately regulated. Each of the two metabolites functions as an intercellular signal, inducing the expression of genes responsible for its own biosynthesis. Our previous study indicate that phloroglucinol, an intermediate in the synthesis of 2,4-DAPG, plays an important role in the regulation of the transcription of the pyoluteorin biosynthetic genes. But the mechanism of phloroglucinol in the regulation of pyoluteorin biosynthesis is still unclear. Here we report that phloroglucinol-mediated positive regulation of the pyoluteorin biosynthesis gene *pltA* by the linked transcriptional regulator PltR was abolished by a mutation in *pltM*, which encodes a putative halogenase. A *pltM* mutant of Pf-5 did not produce pyoluteorin or express *pltA*, even in the presence of phloroglucinol. Purified PltM converted phloroglucinol into a new compound *in vitro*. This compound remarkably induced *pltA* expression in a *pltM* deficient strain. We are currently working on the chemical structure of the compound by HPLC-MS.

Poster #165

Comparative analysis of oomycete diversity between water and sediment in a runoff water sedimentation reservoir in Virginia

XIAO YANG, Chuanxue Hong

Virginia Tech, Virginia Beach, VA USA

Agricultural runoff sedimentation reservoirs are an emerging aquatic system of critical importance to plant biosecurity, water and environmental sustainability. Surveys have been intensified to assess risks to ornamental crops posed by oomycete plant pathogens in recycling irrigation systems. In this study, oomycete communities in a sedimentation reservoir of ornamental plant nursery were determined using a direct plating method. Sediments samples were taken from five depths of 0, 0.76, 1.4, 1.8 and 2.4 m from the surface in February, 2011 and March, 2015. Three *Phytophthora* species including *P. nicotianae*, *P. pini*, and *P. tropicalis* were recovered from sediment surface, approximately 20 *Pythium* and *Phytophythium* species including plant pathogens such as *Py. acanthicum*, *Py. dissotocum*, *Py. irregulare*, *Ph. helicoides*, and *Ph. vexans* were recovered from depths 0 and 0.76 m. No oomycete was recovered from deeper sediments. Additionally, DNA was extracted from the sediment samples as well as water samples and these DNA extracts are being analyzed using next-generation sequencing technology to reveal the oomycete diversity in this sedimentation reservoir.

Poster #166

Estimating the functional potential of *Arabidopsis thaliana* root endophyte communities

SCOTT YOURSTONE  Corbin Jones, Sur Herrera Paredes, Hunter Cameron, Jeff Dangl

University of North Carolina, Chapel Hill, NC USA

Derek Lundberg

Max Planck Institute for Developmental Biology, Tübingen Germany

Scott Clingenpeel, Tanja Woyke, Susannah Tringe

Joint Genome Institute, Walnut Creek, CA USA

We designed and are now testing microbial consortia, guided by the prediction that the level of metabolic complementarity within the plant microbiome is predictive of the level of mutually beneficial interactions, and thus, of microbiome productivity. The productivity of a beneficial plant microbiome, should, in turn, increase plant productivity. In order to test this hypothesis, a diverse library of 200 genome-sequenced bacterial strains isolated from *Arabidopsis thaliana* roots is being used. Bacterial consortia were constructed in a way that maximizes the ranges of metabolic complementarity, as defined below. An array of gnotobiotic *A. thaliana* were inoculated with these consortia and plant growth and transcriptional profiles are being measured. Linking genome-derived predictions of community function to measurable phenotypes will help us infer design principles that will be applied first in controlled settings and ultimately in field settings.

Poster #167

Root Class Specific Association of Soil Microbial Populations

RICHARD ZOBEL

USDA-ARS-PSRU, Raleigh, NC USA

There has been a paucity of research on specific interactions between soil microbial populations and plant roots. A complicating aspect of these interactions is the recently documented presence of 6 or more developmentally and functionally distinct classes of root in a given mature plant root system. Additionally, much of the laboratory level research on plant roots and their interactions has been done with seedlings which only express 3 to 4 of these root classes. We carried out 3 experiments using 5 different perennial ryegrass (*Lolium perenne* L.) clones grown in potted native and artificial soils amended with unsterilized agroforestry pasture soil. Roots of 4-week-old transplants were removed from the soil and the rhizosphere (soil particles and included micro-organisms), from two classes of root washed into tubes and prepared for biologic analysis. The biologic analyses of the rhizosphere of 4 of the 5 clones demonstrated that the microbial populations of shoot-borne (tiller) roots were distinct from populations washed from their attached lateral roots. All five clones demonstrated distinctly different lateral/parent biologic patterns. In one experiment, the clones were watered with leachates from dried leaves of four different species of tree, or rainwater. Each type of leachate differentially affected the lateral/parent biologic patterns. We conclude that soil microbial population distributions in the rhizosphere are normally plant species, cultivar and root class specific. We also conclude that these patterns can be differentially affected by the specific root-zone environment.



An effort to increase interest and funding in phytobiomes research and application of the knowledge to promote food, feed, and fiber production.

phytobiomes.org
[@phytobiomes](https://twitter.com/phytobiomes)