2019 Southern Division Meeting Abstracts (Part I)

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[2019 Southern Division Meeting Abstracts \(Part II\)](#)

Comparative transcriptome analysis between a resistant and a susceptible wild tomato accession in response to *Phytophthora parasitica*

Zunaira Afzal and Gul Shad Ali, University of Florida, Apopka, FL, USA

Phytophthora parasitica is one of the most widespread *Phytophthora* species, which is known to cause multiple diseases in tomato. Our current understanding of tomato-*Phytophthora parasitica* interaction is very limited and currently nothing is known at the whole genome or transcriptome level. In this study, we have analyzed and compared the transcriptome of a resistant and a susceptible wild tomato accession in response to *P. parasitica* infection using the RNA-seq technology. We have identified 2657 and 3079 differentially expressed genes (DEGs) in treatment vs control comparison of Sp-R and Sp-S samples respectively. Functional annotation of DEGs revealed substantial transcriptional reprogramming of diverse physiological and cellular processes particularly the biotic stress responses in both Sp-R and Sp-S upon *P. parasitica* treatment. However, subtle expression differences among some core plant defense related genes were identified and their possible role in resistance development against *P. parasitica* is discussed. Our results revealed 1173 genes that were differentially expressed only in Sp-R accession upon *P. parasitica* inoculation. These exclusively found DEGs in Sp-R accession included some core plant defense genes for example, several protease inhibitors, chitinases, defensin, PR-1, a downy mildew susceptibility factor etc. were all highly induced. Whereas, several R genes, WRKY transcription factors and a powdery mildew susceptibility gene (*Mlo*) were highly repressed during the resistance outcome. Analysis reported here lays out a strong foundation for future studies aimed at improving genetic resistance of tomato cultivars against to *Phytophthora* species.

Population distributions and densities of plant parasitic nematodes in Tennessee and Kentucky field crops

Rufus Akinrinlola, Univ. of Tennessee Knoxville, Jackson, TN, USA and Heather M. Kelly, Univ. of Tennessee, Jackson, TN, USA

Plant parasitic nematodes cause damage and yield loss to crops worldwide. In Tennessee, the most recent study on these parasites was conducted more than two decades ago. In 2018, a soil survey campaign was conducted to determine the population distributions and densities of major plant parasitic nematodes in row crop fields in Tennessee and Kentucky. Additional information, such as soil type and crop rotation were also collected to investigate correlations between nematode results. The information obtained from the study will aid in recommending management tactics to county extension agents and growers. Soil samples were collected from over 80 different fields across eleven counties in Tennessee and 6 counties in Kentucky. Each sample, at 100 cc, was processed for the presence of plant parasitic nematodes. Spiral, stunt, soybean cyst (SCN), and root-knot nematodes were the most predominant nematodes found in the samples, with their population occurring in 88, 47, 47 and 12% of the samples, respectively. Their population densities, juveniles in 100 cc of soil, ranged from 7 to 306 for spiral nematode, 7 to 69 for stunt, 23 to 446 for root-knot, and 115 to 4,038 (eggs) for SCN.

Additional samples and data are being processed and analyzed, including HG type testing of SCN. Total results and analyses from 2018 will be presented. At this time, growers with significant nematodes should consider developing appropriate preventive strategies to limit nematode populations increase and spread that can cause potential crop damage and yield loss.

Virus community analysis to identify the underlying structure of plant-virus interactions

Ricardo Ivan Alcalá-Brise, University of Florida, Gainesville, FL, USA and Karen A. Garrett, Plant Pathology Department, University of Florida, Gainesville, FL, USA

The complexity of plant-virus interactions in ecological landscapes motivates new analytical approaches, such as network analysis of plant-virus associations. Bipartite networks - networks with two types of nodes - have proven useful for understanding plant-pollinator and host-parasitoid interactions. Bipartite networks can be applied more widely to identify generalist and specialist pathogens and their potential threat to new host species. We assembled data from previous studies and public databases of plant virus communities and analyzed bipartite networks describing their associations. Datasets from agroecological landscapes revealed both nested patterns (in which communities tend to include subsets of virus species from other communities) and modular patterns (in which there are distinct sets of associated virus species and hosts). Analyzing the community structure of plants and their associated viruses is a first step toward understanding the underlying processes that drive community assemblage. For example, in analyses of the papaya orchard virome, we found that weeds and insects shared some virus species with papaya, and the patterns of virus species sharing differed across geographic regions. We evaluate what other forces shape viral community structure, comparing communities of viruses from temperate versus tropical regions, from monocot versus dicot hosts, and from natural versus agricultural areas.

Integrating data sources to forecast risk of cassava mosaic disease, an emerging threat in Southeast Asia

Kelsey F. Andersen¹, Erik Delaquis², Cu Thi Le Thuy³, Nami Minato², James Peter Legg⁴ and Karen A. Garrett¹, (1) Plant Pathology Department, University of Florida, Gainesville, FL, USA, (2) International Center for Tropical Agriculture (CIAT), Vientiane, LAOS, (3) International Center for Tropical Agriculture (CIAT), Hanoi, Vietnam, Hanoi, VIETNAM, (4) IITA, Dar Es Salaam, TANZANIA

Cassava mosaic viruses, causal agents of cassava mosaic disease (CMD), are a major threat to cassava yield globally. *Sri Lankan cassava mosaic virus* (SLCMV) was first reported in Cambodia in 2015 and more recently in Vietnam. It is vectored by whiteflies and spread by infected cassava planting material. We present a computational framework for combining social and environmental geospatial data layers in a multilayer network model, to forecast and map regional disease risk and outcomes of management scenarios. Data for parameterizing the farmer trade network model include household surveys conducted in Cambodia and Vietnam in 2016 and 2017, and associated surveys of SLCMV incidence. We used exponential random graph models (ERGMs) to model likely SLCMV regional dispersal routes. We identified priority locations both for pathogen surveillance and for epidemic management (through phytosanitary regulation, deployment of new varieties, farmer education). Interestingly, locations that were optimal for surveillance were not necessarily optimal for management, a practical finding for epidemic mitigation. We present potential patterns of introduction and spread of SLCMV, regional risk maps, and management recommendations from scenario analysis. Additionally, we discuss risk pathways what may be important for the introduction of additional strains of cassava mosaic virus and other cassava pathogens.

Development of four Locked Nucleic Acid-based real-time PCR assays for early detection of turfgrass root-infecting pathogens

Aline Badial, Matthew Aaron Tucker, Jonas King and Maria Tomaso-Peterson, Mississippi State University, Mississippi State, MS, USA

Ectotrophic root-infecting (ERI) fungi comprise several genera of soilborne pathogens frequently associated with bermudagrass putting greens (BPG). Early and accurate detection of ERI fungi are strategic practices to minimize fungal colonization and disease development in BPG. In this study, we developed four novel Locked Nucleic Acid (LNA)-based real-time PCR assays to detect four ERI fungi known to cause root rot in BPG. Probes and primers were developed to detect *Gaeumannomyces graminis*, *Gaeumannomyces* sp., *Magnaportheopsis cynodontis*, and *Candidacolonium cynodontis*. Validation of the primers and probes was conducted using DNA from each fungus and PCR efficiencies were calculated for each assay. Cross-validation experiments were also performed by spiking DNA from non-target ERI fungi in PCR reactions to confirm the specificity of the assays. Our results showed that the developed LNA assays successfully distinguished all four fungi in infected bermudagrass root samples. No amplification was detected in reactions using non-target DNA samples, indicating 100% specificity of the optimized assay. The limit of detection was established to be at 1 pg/μL of DNA per reaction proving the sensitivity of the method to detect very low amounts of the target fungal DNA in bermudagrass roots. The LNA-based real-time PCR probes developed in this study are a reliable method for the rapid and accurate detection of the four ERI fungi associated with root rot of BPG.

Control of Phytophthora root rot and ambrosia beetles on flowering dogwood trees during simulated flood events

Fulya Baysal-Gurel, Matthew Brown, Jason B. Oliver and Karla Adesso, Tennessee State University, McMinnville, TN, USA

Phytophthora root rot causes major economic losses in woody ornamental nurseries, especially in plants exposed to elevated soil moisture levels. Ambrosia beetles, which attack stressed trees, are also important pests of woody plants. In this study, several products were evaluated for control of Phytophthora root rot and ambrosia beetles on containerized flowering dogwoods exposed to simulated flood events. Longer flooding duration increased disease pressure from *Phytophthora cinnamomi*. Preventive treatment of plants with Subdue MAXX consistently reduced disease pressure at tested flooding durations of 1, 3, or 7 days. Preventive and curative treatments of Empress Intrinsic, Orkestra Intrinsic and Pageant Intrinsic were effective at 1 and 3 days of flooding. Preventive RootShield Plus⁺ treatment was effective on plants flooded 1 day. *Phytophthora* inoculation increased ambrosia beetle attacks on flooded dogwoods, but preventively-applied Orkestra Intrinsic, Pageant Intrinsic and Subdue MAXX, and curatively-applied Orkestra Intrinsic and Tartan

Stressgard reduced ambrosia beetle attacks compared to non-treated, inoculated controls. Preventively-applied Orkestra Intrinsic, Pageant Intrinsic and Subdue MAXX and curative treatment of Orkestra Intrinsic were effective at reducing both *Phytophthora* and ambrosia beetle damage. These results can help nursery producers successfully manage *Phytophthora* root rot and also reduce the risk of ambrosia beetle attacks during flood events.

Management of Fusarium head blight of sorghum in North Carolina

Jean Beacorn, North Carolina State University, Raleigh, NC, USA

Sorghum (*Sorghum bicolor*) in North Carolina is primarily grown for food or animal feed. Growing sorghum in this region is challenged by environmental conditions conducive for disease infection and development by mycotoxigenic fungi, specifically for fungi associated with the Fusarium Head Blight (FHB) disease complex. There are few management strategies available for FHB in sorghum, and timing of fungicide applications is not well understood. Our goal is to evaluate the efficacy of four fungicides at three application timings. The fungicides prothioconazole (Proline), prothioconazole + trifloxystrobin (Delaro), fluopyram + trifloxystrobin (Luna Sensation), and prothioconazole + tebuconazole (Prosaro) were applied at 20 gal/A at 15psi at heading, anthesis and soft-dough stages of sorghum growth. High precipitation in early season, Hurricane Florence, and Hurricane Michael were environmental factors that potentially affected the results of this experiment. Luna Sensation at soft-dough provided the lowest average disease severity with 19.87% severity. Delaro at soft-dough provided the highest average yield at 9.54 bu/acre ($P = 0.03$). Future experiments will include replicated field experiments and evaluation of mycotoxins for deoxynivalenol (DON), fumonisin, and zearalenone to determine optimal fungicide and timing for FHB management.

Characterization of resistance to *Ustilago maydis* in teosinte and two maize-teosinte introgressed near isogenic lines

Usha Bhatta, The University of Georgia, Athens, GA, USA and Shavannor M. Smith, University of Georgia, Athens, GA, USA

Ustilago maydis (*U. maydis*), a causal agent of corn smut, is an important agricultural pathogen. The pathogen is responsible for significant economic loss of around \$1 billion annually in the United States. The lack of resistant maize cultivars warrants the need for new sources of resistance to *U. maydis*. We identified two near isogenic lines (NILs) from a maize (B73)-teosinte (*Zea parviglumis*) introgression population that are resistant to *U. maydis*. Genotypic and phenotypic analysis of the two resistant NILs identified a 3.6 Mbp teosinte introgression region on the short arm of chromosome 9, indicating that the teosinte introgressed regions was responsible for the resistance phenotype. Teosinte has not been sequenced. Therefore, comparative analysis of the B73 maize reference genome and the two resistant NILs identified seven protein kinase genes on the short arm of chromosome 9 in maize, collinear to the teosinte introgressed regions in the two NILs. For this study, we aim to 1) Characterize the seven-protein kinase R-genes (resistance genes) in the resistant NILs and teosinte lines, 2) Sequence the *Zea parviglumis* genome to identify additional R-genes in the 3.6 Mbp region, and 3) Conduct quantitative real time PCR to select the expressed R-genes in the introgressed region. Needle inoculation of *U. maydis*, in addition to PCR based cloning and sequencing of the seven R-genes was performed in B73, NILs and the teosinte lines. The phenotypic data supports the genotypic data indicating the resistance in the NILs is due to the *Zea parviglumis* introgression. Whole genome sequencing of the teosinte parent is ongoing and will identify R-genes and other defense related genes in the NILs and teosinte that confer resistance to *U. maydis*.

RNA-Seq analysis and de novo transcriptome assembly of *Phytophthora parasitica* treated with silver nanoparticles

Shaheen Bibi¹, Ashraf El-Sayed², Jose C. Huguet-Tapia¹ and Gul Shad Ali², (1) University of Florida, Gainesville, FL, USA, (2) University of Florida, Apopka, FL, USA

Phytophthora parasitica is a soil-borne pathogen and infects a wide range of plant species including many economically important crops. Fungicides have been used since long time for controlling the diseases caused by *P. parasitica*. However, for sustainable management, it is essential to discover new effective control methods to control this notorious pathogen and the diseases. Aside of using chemical, many studies have reported effective biological methods to control the disease. We have indicated the effectivity of using silver nanoparticles to control the disease in vivo and in vitro in previous studies. In order to gain the insight into the various biological pathways effected by treatment with nanoparticles, we have conducted a transcriptome analysis of *P. parasitica* using the de novo approach and reference to the genome. The analysis has revealed that expression level of many genes is modulated using silver nanoparticles. Gene ontology analysis of the differentially expressed genes suggested that these genes are involved in cell wall synthesis and amino acid metabolism. Finding of our analysis using two different approaches lays foundation to future analysis of using silver nanoparticles to control the disease and indicated that silver nanoparticles interfere with *Phytophthora parasitica* growth affecting many essential biological processes.

Improved annotation of the nucleotide-binding domain leucine-rich repeat containing NLR gene family in citrus

Qiaolin Zheng¹, Marco Pitino¹, Surya Saha², Mirella Flores², Prashant Hosmani², Lukas Mueller² and Liliana M. Cano¹, (1) University of Florida, Fort Pierce, FL, USA, (2) Boyce Thompson Institute, Ithaca, NY, USA

One of the major diseases in citrus is caused by the fastidious bacteria Candidatus Liberibacter asiaticus. There are efforts towards finding resistant cultivars that could effectively mitigate these losses and control other citrus bacterial and fungal diseases. Resistant

cultivars contain plant resistance (*R*) genes which encode immune receptors composed of nucleotide-binding and leucine-rich repeat (NLR) domains. These NLR proteins mediate the specific recognition of pathogen avirulence effectors to induce defence responses. In this study we performed a search for NLRs using NLRparser software which uses a predefined biologically curated motif composition database. We identified a NLR complement set of about 600 NLR proteins using as reference the publicly available predicted proteomes of *Citrus sinensis* and *Citrus clementina*. We are currently performing annotations from the known tolerant *Poncirus trifoliata* using 6-frame translations from an EST database and also from the proteomes of two non-host plants that exhibit citrus greening disease symptoms, periwinkle *Catharanthus roseus* and *Nicotiana benthamiana*. Our goal is to contribute to the citrusgreening.org community with an improved annotated NLRome dataset which can be used in comparative analysis between susceptible and potentially tolerant citrus species.

Bactericidal activity of Copper-Zinc Hybrid Nanoparticles on *Xanthomonas perforans* and effect on Xanthomonadin

Renato Carvalho, University of Florida, Gainesville, FL, USA, Mathews L. Paret, University of Florida, Quincy, FL, USA, Jeffrey B. Jones, Department of Plant Pathology, University of Florida, Gainesville, FL, USA and Kamil Duman, University of Florida - NFREC, Quincy, FL, USA

Bacterial spot of tomato, caused by *Xanthomonas perforans*, *X. euvesicatoria*, *X. vesicatoria* and *X. gardneri* is a major devastating disease, contributing to significant yield losses in tomato production in the U.S. In Florida and other southeastern states the conditions are ideal for bacterial spot where warm and wet weather conditions are present around the year. Over dependence of conventional copper bactericides over the last many decades have led to the development of copper tolerant strains of *Xanthomonas* spp., making copper bactericides ineffective. Thus there is a critical necessity to develop new strategies to manage copper tolerant *Xanthomonas* spp. In this study we evaluated the antibacterial potential of a Cu/Zn hybrid nanomaterial in-vitro and in planta. Raman spectroscopy was used to study the action of the Cu/Zn on a key bacterial pigment xanthomonadin. In vitro tests indicate the potential of Cu/Zn to prevent bacterial growth. In vivo tests showed the potential of Cu/Zn to significantly decrease bacterial spot disease severity compared to the water control and grower standard (copper+mancozebe). There was no phytotoxicity on plants due to application of Cu/Zn. Bacterial cells exposed to Cu/Zn had significantly reduced amounts of xanthomonadin. These preliminary results indicate that Cu/Zn could be a potential strategy to manage bacterial spot on tomato. Further field studies are in progress to understand field level efficacy of this hybrid nanomaterial.

Perfect storms: Regional risk to agriculture from extreme weather events

Robin Choudhury and Karen A. Garrett, Plant Pathology Department, University of Florida, Gainesville, FL, USA

Extreme weather events, like hurricanes, droughts, and resulting fires, can disrupt agriculture across regions and countries. These events not only damage the infrastructure necessary to mitigate plant disease epidemics, but can also increase the risk of introduction and spread of new pathogens and pests. Our objectives were to identify plant disease epidemic risk factors associated with extreme weather events and to use county-level data to assess which agricultural regions of the US were most at risk, with a focus on the Southeast. Wind- and water-borne dispersal of pathogen propagules, which can be accelerated by extreme weather events, can rapidly disseminate pathogens and pests into new regions. Extreme weather events can also have short-term effects on host stress, which can severely affect the long-term health of plants, especially in perennial cropping systems. Increased importation of raw materials post-storm can introduce new pathogens, but effective monitoring and sanitation of materials can mitigate this risk. We found that agriculture in coastal, low-lying areas of the southeastern US is at particular risk from direct storm damage. Furthermore, this risk may be elevated by aggregation of particular crops within a region, where introduced pathogens can more rapidly spread among nearby hosts. We discuss these findings in the context of climate change and increased frequency of damaging storms.

Current production practices, level of risk, and spotted wilt disease intensity in commercial peanut fields in Georgia

Clarence Codod¹, Robert Kemerait¹, Albert K. Culbreath¹, Mark Abney² and George G. Kennedy³, (1) UGA Dept of Plant Pathology, Tifton, GA, USA, (2) UGA Dept of Entomology, Tifton, GA, USA, (3) North Carolina State University, Raleigh, NC, USA

This study aimed to document the growers' production practices and assess the level of risk as well as spotted wilt disease intensity in commercial peanut fields across the peanut production region of Georgia. Spotted wilt intensity ranged from 0.25 to 39.25%, with a mean of 3.66%, in 73 fields surveyed in 2017. In 2018, it ranged from 0.13 to 53.13% (mean=4.29%) in 83 fields. Majority of the fields were planted with GA-06G variety in both years. Most peanut fields were planted between May 1-10 (28.77 and 23.7%) and May 11-31 (45.21 and 27.7%) in 2017 and 2018, respectively. Phorate insecticide was applied in more than 50% of the surveyed fields. The other fields had either imidacloprid or nothing. Conventional tillage was widely practiced with either single or twin row pattern. Consistent with previous reports, higher spotted wilt intensity was observed on peanuts planted before May with conventional tillage, single row pattern, and where phorate insecticide was not applied. Peanut Rx risk values, based on production practices and inputs, ranged from 40 to 90 total points in both years. Positive linear relationship was detected between observed spotted wilt and Peanut Rx risk points. None of the fields with low risk values had high disease intensity, while fields with the highest risk values had the highest spotted wilt intensity. These fields were located in Irwin and Tattnall county (2017) and Worth and Appling county in 2018. This study provides information on current production practices and spotted wilt intensity in commercial fields, which are useful in directing future research projects to benefit the growers.

Banana and plantain disease risk in Haiti: Diagnostic networks, seed networks, and cropland connectivity

Wanita Dantes^{1,2,3}, Joubert Fayette^{1,2,3}, James Fulton^{1,2,3}, Yanru Xing^{1,2,3}, Kelsey F. Andersen^{1,2,3}, Margareth Divers^{1,2,3} and Karen A. Garrett^{1,2,3}, (1) Plant Pathology Department, University of Florida, Gainesville, FL, USA, (2) Institute for Sustainable Food Systems, University of Florida, Gainesville, FL, USA, (3) Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA

Banana and plantain are important parts of the diet in Haiti and are subject to yield loss from several important pathogens. We evaluate three key elements of disease management strategies in Haiti and how they interact. (1) Plant diagnostic networks are vital components of plant protection services. The national disease reporting by the Ministry of Agriculture has decreased significantly over the last decades. We analyze the national network of diagnostics, including the Ministry of Agriculture, diagnostics expertise across the country, and plant disease clinics. (2) Seed networks for the distribution of banana planting material make new varieties available and are a risk factor for the spread of pathogens. We present a preliminary representation of banana seed networks and their potential role in the spread of disease in Haiti. (3) Cropland connectivity analyses represent the disease risk due to spread between plantings of banana and plantain, and other species that share the same pathogens. Plantings that are closer together, and that have denser production of banana and plantain, have a greater risk of spread. Haiti has particularly dense plantings of banana and plantain for the Caribbean region, and so is at high risk of pathogen introduction and establishment, and of being a source of pathogens. We evaluate how these system components interact to create higher and lower disease risk regions for banana and plantain in Haiti.

Identification of *Xylella fastidiosa* subsp. *fastidiosa* from infected southern highbush blueberry (*Vaccinium* sp.) in Georgia

Dario Di Genova, Kippy Lewis and Jonathan E. Oliver, University of Georgia, Tifton, GA, USA

Bacterial Leaf Scorch (BLS), caused by *Xylella fastidiosa* (Xf), is a significant threat to blueberry production in the southeastern U.S. Xf consists of multiple genetically distinct subspecies that differ in virulence and host range. All previous isolations from naturally-infected blueberries showing BLS symptoms have identified Xf subsp. *multiplex* (Xfm) as the cause of BLS. In fall 2017, multiple plantings of southern highbush blueberries (interspecific *Vaccinium corymbosum* hybrids) in southern Georgia showing typical BLS symptoms were surveyed, and seven Xf isolates were cultured from collected symptomatic leaves. PCR amplification and sequencing of a portion of the Xf *rpoD* gene revealed unexpected genetic differences amongst the seven isolates. Utilizing a multilocus sequence-typing approach, three of the seven isolates from blueberry were classified genetically as Xf subsp. *fastidiosa* (Xff), with high similarity to sequences from isolates causing Pierce's disease of grapevine. Inoculation of greenhouse-grown blueberries (cv. 'Rebel') with these Xff isolates from blueberry led to the development of typical BLS symptoms. Five months post-inoculation, the presence of Xff in the inoculated plants was confirmed by re-isolation, PCR analysis, and sequencing. These findings suggest that both Xff and Xfm isolates can infect and cause leaf scorch symptoms on blueberry, which has potential implications for Xf evolution and BLS management in blueberries.

Managing bacterial spot of pepper caused by *Xanthomonas euvesicatoria* using novel copper-composites

Qiurong Fan¹, Ying-Yu Liao², Sanju Kunwar², Mikael Young³, Swadeshmukul Santra⁴, Jeffrey B. Jones⁵ and Mathews L. Paret¹, (1) University of Florida, Quincy, FL, USA, (2) University of Florida, Gainesville, FL, USA, (3) NanoScience Technology Center and Burnett School of Biomedical Science, UCF, Orlando, FL, USA, (4) University of Central Florida, Orlando, FL, USA, (5) Department of Plant Pathology, University of Florida, Gainesville, FL, USA

Bacterial spot disease of pepper caused by *Xanthomonas euvesicatoria* (Xe) leads to significant crop losses worldwide. The efficacy of copper bactericide application for managing bacterial spot, has been limited by the widespread occurrence of copper tolerant Xe strain. In this study, we investigated the antibacterial effect of three novel copper-composites, Core-shell copper (CS-Cu), Mixed-valence copper (MV-Cu), and Fixed-quat copper (FQ-Cu) in *in vitro* and in greenhouse experiments against Xe copper tolerant strains. All concentrations (100, 200, 500 and 1000 µg/ml) of CS-Cu and FQ-Cu effectively killed bacteria within 1 h and MV-Cu killed all the cells within 24 h, *in vitro*. In the first greenhouse experiment, plants treated with all copper-composites, at all concentrations (100, 200, and 500 µg/ml) had disease severity (AUDPC) statistically equivalent to or better than copper-mancozeb, the growers' standard. All three concentrations of FQ-Cu, 200 and 500 µg/ml of CS-Cu and 100 and 200 µg/ml of MV-Cu showed significantly less AUDPC compared to Kocide 3000. In the second greenhouse experiment all concentrations of FQ-Cu and 100 µg/ml, 200 µg/ml of MV-Cu significantly reduced AUDPC compared to Kocide-3000. All concentration of MV-Cu and FQ-Cu were statistically equivalent to copper-mancozeb. FQ-Cu and MV-Cu with low-to-no phytotoxicity and consistent efficacy highlight the potential of copper-nanocomposites for effective management of bacterial spot of pepper.

An emerging plantain soft rot caused by *Klebsiella variicola*

James Fulton¹, Sladana Bec², Joubert Fayette¹, Karen A. Garrett¹, Carrie Lapaire Harmon³ and Randy C. Ploetz⁴, (1) Plant Pathology Department, University of Florida, Gainesville, FL, USA, (2) University of Florida, Gainesville, FL, USA, (3) Univ of Florida PDC, Gainesville, FL, FL, USA, (4) University of Florida, Homestead, FL, USA

In February 2018, we investigated reports of a possible new disease affecting plantain in the Ouest department of Haiti. Plants from four farms were sampled and bacterial isolates cultured. Symptoms included a chocolatey brown necrotic soft discoloration which extended several feet in length and width while penetrating tissue to a depth of several centimeters. Lodging often occurred at fruit onset when

fruits were near their maximum weight, producing “toppling” symptoms. Field incidence rates were 1–30%, and rates as high as 60% have been reported. Pure bacterial colonies were derived from symptomatic pseudostem sections. The isolates were Gram-negative and formed gray-white, glossy, convex, translucent, and round colonies on NA. Isolates were used to perform pathogenicity tests on disease-free banana plantlets. Rotting symptoms, consistent with the field observations, were observed four weeks after inoculation. Reisolation and identification completed Koch’s postulates. Amplified sequences of 16s ribosomal RNA, the *rpoB* (RNA polymerase beta subunit), *phoE* (outer membrane pore protein E), and *infB* (translation initiation factor IF-2) were used to infer phylogenetic trees. These analyses indicate that the pathogen is *Klebsiella variicola*. There is also potential for these symptoms to represent a disease complex, with other bacterial pathogens and potentially interactions with nematodes or insect pests.

Effectiveness of biological control agents and host resistance for the management of Granville wilt (*Ralstonia solanacearum*) of tobacco

Raymond Garcia and Lindsey D. Thiessen, North Carolina State University, Raleigh, NC, USA

Ralstonia solanacearum affects over 450 plant species including economically important crops such as tomato, potato and tobacco. Management strategies for Granville wilt of tobacco rely on integrated pest management (IPM), which combines multiple control practices. Therefore, to investigate alternative control practices, the efficiency of three biocontrols and compost in combination with two levels of host resistance were evaluated for the management of Granville wilt in a naturally-infested farm in North Carolina. The field trial was transplanted in May and all common agricultural practices outside of disease management were followed throughout the growing season. Biological control agents (BCAs) included Bio-Save (*Pseudomonas syringae*), Serifel (*Bacillus amyloliquefaciens*) and Serenade ASO (*Bacillus subtilis*). McGill compost was incorporated into the beds prior to transplanting and BCAs were applied by soil drenching using a pressurized backpack sprayer. Visible symptoms were used to assess disease incidence at weekly intervals starting 27 days after transplanting and the presence of *R. solanacearum* was confirmed using *R. solanacearum* specific ImmunoStrip®. The treatment combinations ‘NC471’-McGill compost and ‘NC196’-Serifel significantly reduced ($P \leq 0.05$) the final disease incidence percentage when compared to the highest disease control ‘NC196’-Bio-Save. However, there were no significant differences in the final disease incidence percentage amongst these and seven other treatments ($P > 0.05$), including non-treated controls. Lastly, no phytotoxicity nor secondary infections were observed.

Optimal parameters for conditioning sclerotia of Southeastern U.S. isolates of *Sclerotinia sclerotiorum*

Christopher James Gorman, Auburn University, Auburn University, AL, USA and Kira L. Bowen, Auburn University, Auburn, AL, USA

Carinata is a non-food oil seed grown as a summer crop in the Canadian Plains as well as the U.S. North Central states. Products from carinata include biofuels such as drop-in jet fuel, bio-lubricants, high protein meal, and more. There is currently an initiative to introduce carinata into the southeast US as a winter crop. *Sclerotinia* stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is known to cause significant yield losses in oil seed brassicas. As such, preferred environmental conditions of various life stages of Southeastern isolates of *S. sclerotiorum* (from AL, GA, and FL) are being evaluated to modify or validate a disease forecasting system for use in aiding disease management decisions. To date, we have tested environmental parameters for conditioning sclerotia. These parameters included 4 temperature treatments (constant 12°C, and 15/3°C, 18/6°C, 21/9°C day/night), 3 moisture levels based on frequency of events (every 1, 3, or 6 days) and three conditioning durations (4, 6, and 8 weeks). Preliminary results show high variability in responses among isolates based on apothecia produced from sclerotia after conditioning, with most isolates able to germinate at all of the temperature-moisture-duration treatments. The exception to this was that no isolate was able to germinate after being conditioned for 6 or 8 weeks with a moisture event every day at 21/9°C.

An evaluation of plant-growth promoting rhizobacteria for management of *Meloidogyne incognita* on turfgrass

Will Groover¹, Kathy S. Lawrence², David Held² and Kendra Carson², (1) Auburn University, Auburn University, AL, USA, (2) Auburn University, Auburn, AL, USA

Meloidogyne incognita, root-knot nematodes, are among the most economically important plant-parasitic nematodes in turfgrass in the southeastern United States. Only a few nematicides are available or efficacious for plant-parasitic nematodes in turfgrass, and recent work in our lab has demonstrated the potential for microbial control of root-knot nematodes in field crops. The objectives of this study are to evaluate the ability of 104 plant growth-promoting rhizobacteria (PGPR) strains isolated from grasses in Alabama for their mortality of *M. incognita* in-vitro, and their ability to manage plant-parasitic nematodes in the greenhouse setting. Strains were evaluated in-vitro in 96-well plates, with mortality from each strain compared to non-treated and chemical nematicide, fluopyram, after 24, 48, and 96 hours. Of these 104 strains, 51% are *Bacillus* spp., 25% are other genera, and 24% are still unknown as identification is still ongoing. In-vitro mortality ranged from 2% to 93%, with an average mortality of 22%. Based on the in-vitro analysis, 11 strains were selected for further screening in a turfgrass system. These include 7 *Bacillus* spp., 1 *Comamonas* sp., 1 *Gordonia* sp., 1 unknown species, and Blend 20, a blend of three *Bacillus* strains previously found to enhance growth and drought tolerance in grasses. Greenhouse evaluations have begun, with trial completion occurring mid-January. Microplot and field analysis are scheduled for spring and summer of 2019.

The effects of *Soybean vein necrosis virus* on soybean yield in West Tennessee

Rachel Guyer, Larissa Visioli, Jonathan Lacey and Heather M. Kelly, University of Tennessee, Jackson, TN, USA

Soybean (*Glycine max*) is a crop of global importance and the U.S. is the world's largest soybean producer. Several viruses can infect soybean and result in significant yield loss. *Soybean vein necrosis virus* (SVNV), a tospovirus, was first reported in Tennessee in 2008, and has since been found in 12 additional states: Alabama, Mississippi, Arkansas, Oklahoma, Kansas, Missouri, Kentucky, Ohio, Iowa, Wisconsin, Illinois, and Michigan. SVNV symptoms were observed in West Tennessee in the 2018 growing season, which was the first time it had been observed in such abundance since 2012. This study aimed to: identify and collect symptomatic and asymptomatic plants; confirm virus presence by ELISA; and evaluate and compare yield parameters of sampled plants. Symptomatic and asymptomatic plants were identified and tagged in five experimental field trials across two locations in West Tennessee: Milan and Jackson. From each sampled plot, paired asymptomatic and symptomatic leaf samples were taken at growth stages R5 and R6, and whole plants were hand harvested at growth stage R8. Five yield parameters were recorded: plant height, number of pods per plant, number of seeds per pod, plant yield, and 100-seed weight per plant. Presence of SVNV was detected by an ELISA test in all the symptomatic samples across both locations and all asymptomatic samples were negative, except one sample from Jackson. None of the yield parameters of the symptomatic plants were significantly different from those of the asymptomatic plants.

The effect of exogenous pectin amendments on soybean growth-promotion by PGPR

Mohammad Hassan, John A. McInroy, Deepak Shantharaj, Mark Liles and Joseph W. Kloepper, Auburn University, Auburn, AL, USA

The efficacy of exogenous pectin and orange peel (OP) amendments on soybean plant growth-promotion and nodulation mediated by plant growth promoting rhizobacteria (PGPR) *Bacillus velezensis* (Bv) strains were studied in the greenhouse. The overall hypothesis tested is that the pectin and OP will increase Bv-mediated soybean plant growth-promotion and nodulation. The experimental design included soybean seeds planted in collected field soil that contained commercial *Bradyrhizobium japonicum* inoculant, Bv PGPR strains AP136, AP193, and AP203 with or without pectin (1 mg) and OP (1 & 10 mg) amendments, along with an untreated control. The data on the shoot and root length, dry weights, and nodules were analyzed for statistical significance at 35 days after inoculation (DAI). The results suggested that pectin amendment by Bv PGPR strain AP136 significantly increased (35.52%) shoot length compared to AP136 without pectin. The average soybean nodules and dry weight per plant of Bv PGPR strain AP193 with pectin significantly increased (42.82% and 50.00%) compared to AP193 without pectin. The dry root weight of the plants treated by Bv PGPR strains AP193 and AP203 with OP amendment (10 mg) significantly increased (55.55% and 52.38%) compared to the same PGPR strains without OP amendments. The average number of soybean nodules per plant of Bv PGPR strain AP203 with OP amendment (10 mg) were significantly enhanced (80.88%) compared to the same strain without OP amendment. These studies revealed that co-application of selected Bv PGPR strains with pectin or OP amendment can promote soybean plant growth and nodulation.

Assessment of root protection by fluopyram-treated seed against *Meloidogyne incognita*

Tracy Hawk, Fayetteville, AR, USA and Travis R. Faske, University of Arkansas, Lonoke, AR, USA

The southern root-knot nematode, *Meloidogyne incognita*, is one of the most important plant-parasitic nematodes that affect cotton and soybean production in the southern United States. Nematicides are part of an integrated management system and seed-applied nematicides are among the most commonly used in row crop production. Fluopyram was recently registered for use as a seed-applied nematicide in cotton and soybean, however, the degree of root protection provided by seed-applied fluopyram has yet to be determined. The objective of this study was to determine the effect seed-applied fluopyram on *M. incognita* penetration, infection, and reproduction on cotton and soybean. Fluopyram-treated soybean and cotton seeds were planted in sandy soil infested with the second-stage juveniles (J2) as two separate greenhouse experiments. Abamectin and non-nematicide-treated seed served as industry standard and negative controls, respectively. Roots were sampled weekly to assay nematode infection and reproduction. Fewer J2, galls, and reproduction as eggs were observed with fluopyram-treated cotton and soybean seed compared to non-nematicide-treated seed. Similar results were observed with abamectin-treated cotton and soybean seed. These data suggest that fluopyram as a seed treatment has the greatest impact on J2 infection which reduces subsequent nematode infection and reproduction to a similar degree as that of abamectin.

New insights into the disease cycle of orange cane blotch of blackberry and its management using potassium phosphite

Will Hemphill¹, Phillip M. Brannen² and Jonathan E. Oliver¹, (1) University of Georgia, Tifton, GA, USA, (2) University of Georgia, Athens, GA, USA

Expansion of commercial blackberry production into the warm, humid southeastern U.S. has resulted in the emergence of diseases such as orange cane blotch (OCB), caused by the parasitic alga *Cephaleuros virescens*. This pathogen forms orange sporulating spots on the surface of blackberry canes that reduce blackberry yields. The OCB disease cycle on blackberry is poorly understood, and management options are limited. Application of potassium phosphite reduces disease severity and improves yields, but little is known about the specific effects of potassium phosphite applications on disease development. To better understand both the OCB disease cycle and how potassium phosphite reduces disease severity, field trials were conducted in three commercial blackberry plantings. Detailed observations of the disease cycle were made on unsprayed plants from October 2017 to November 2018. In addition, blackberry plants at these locations were sprayed every 21 days with potassium phosphite at the max label rate (4 pts/A) from June to November 2018, a

total of eight applications. Disease severity in sprayed versus unsprayed plants was assessed on primocanes through the measurement of spot diameter and total spot numbers. Based on the trial results, OCB is a monocyclic disease on blackberry, and potassium phosphite acts by limiting infections of new primocanes as well as spot development. This further explains how timely potassium phosphite applications reduce primocane infections.

Assessing the stability of reduced sensitivity to fentin hydroxide in the pecan scab pathogen *Venturia effusa*

Kory Herrington, Katherine L. Stevenson, Jeffrey R. Standish and Timothy B. Brenneman, University of Georgia, Tifton, GA, USA

Georgia pecan growers lose millions of dollars annually to pecan scab caused by *Venturia effusa*, the management of which relies heavily on fungicides. In this pathogen, reduced sensitivity to fentin hydroxide (TPTH) has been a historical issue but does not appear to be stable over time. The objectives of this study were to evaluate the stability of reduced sensitivity to TPTH in three commercial pecan orchards by treating trees in large replicated blocks with labeled rates of TPTH or the multi-site fungicide Ziram over two consecutive years. In August of 2017 and 2018, nut scab severity was assessed and leaf samples exhibiting typical sporulating scab lesions were collected for sensitivity testing on media amended with 5.0, 10.0, and 30.0 µg/ml of TPTH. Sensitivity to TPTH differed by year and location but not by the fungicide applied to trees from which samples were collected. Mean sensitivity values on 5.0, 10.0, and 30.0 µg/ml were 76.2, 42.6, and 25.7%; and 94.4, 59.1, and 1.6% in 2017 and 2018, respectively. In both years, Ziram gave significantly better nut scab control than did TPTH. Mean nut scab severity in 2017 for Ziram and TPTH-treated trees was 6.3% and 13.1%, and 17.3% and 19.9% in 2018. These results indicate that the mechanisms affecting the stability of reduced sensitivity to TPTH in *V. effusa* may be independent of fungicide selection pressure.

First report of *Colletotrichum siamense* on *Smilax rotundifolia* in the Southeast United States

Jason Hickman¹, Rosanne Healy², Patricia Salinas Soria², Maria Velez-Climent² and Brantlee Spakes Richter², (1) University of Florida Department of Plant Pathology, Gainesville, FL, USA, (2) University of Florida, Gainesville, FL, USA

A survey for plant pathogens on wild plants was conducted in the winter of 2018. *Smilax rotundifolia* was found with leaf spots on the bank of a wooded lake at the University of Florida. In the southeast, *Smilax* is a major food crop for animals. *Colletotrichum* sp. was consistently isolated from lesions, and single spore isolates were established. DNA was extracted and the full ITS region of the rDNA was sequenced. The BLAST return of the sequence against GenBank was a 100 percent match to *Colletotrichum siamense*, a known blueberry pathogen. *Smilax* spp. are known to invade blueberry fields. The following was carried out to test the potential for crossover infection. Isolates of *C. siamense* from *S. rotundifolia* and blueberry were used to inoculate healthy *S. rotundifolia* and blueberry plants. Each set of plants was spray inoculated with each strain of *C. siamense* for an experimental block of 10 plants in each group and 9 controls. At least 7 plants in each block developed lesions. *C. siamense* was re-isolated from lesions, fulfilling Koch's postulates and confirming pathogenicity. A second trial of Koch's postulates was carried out on *S. rotundifolia* with an isolate of *Colletotrichum* from *Smilax bona-nox*. Again, the ITS sequence matched *C. siamense*. A third trial is planned to replicate the experiment on *S. rotundifolia* and blueberry. This is the first report of *C. siamense* on *S. rotundifolia* in the Southeast United States.

Development of novel genetic resources to study *Macrophomina phaseolina* on soybean in Western Tennessee

Jonathan V. Lacey, University of Kentucky, Lexington, KY, USA, Kurt H. Lamour, University of Tennessee, Knoxville, TN, USA and Heather M. Kelly, University of Tennessee, Jackson, TN, USA

Macrophomina phaseolina (Tassi) Gold is the causal agent of charcoal rot in soybean [*Glycine max* (L.)]. In terms of yield loss, charcoal rot is one of the most important soybean diseases in the United States. Five isolates of *M. phaseolina* were recovered from soybean roots located in the Western Tennessee counties of Jackson (N=2), Milan (N=2), and Fayette (N=1). Isolates TN_406 and TN_261 were isolated from Milan, isolates TN_4 and TN_280 were isolated from Jackson and TN_408 was isolated from Fayette County. The isolates genomes were sequenced using an Illumina HiSeq X sequencer and the resulting 150bp paired end reads were aligned to a publicly available *M. phaseolina* reference genome. From the 49.3Mb genome, 70,000 polymorphic single nucleotide polymorphism (SNP) loci were identified. Isolates from Milan were nearly identical with 11 variant alleles and isolates from Jackson were also highly similar with 600 variants, still less than 1% of the genome. Diversity among isolates was high, ranging from 40,000 to 50,000 polymorphic loci when comparing across different field sites. The goal of this work is to identify a subset of neutral SNP loci for fine scale population analyses to conduct further work to better understand the pathogen's survival, spread, and the importance of sexual and asexual reproduction throughout a growing season.

A novel alternative to copper bactericide: Magnesium nano-materials for management of bacterial spot disease of tomato

Ying-Yu Liao¹, Amanda L. Strayer-Scherer², Ziyang Huang³, Swadeshmukul Santra³, Jason C. White⁴, Arnab Mukherjee⁴, Roberto De La Torre-Roche⁴, Qiurong Fan⁵, Susannah Wright⁵, Laura Ritchie⁵, James Colee⁶, Gary E. Vallad⁷, Joshua H. Freeman⁵, Jeffrey B. Jones⁸ and Mathews L. Paret⁵, (1) University of Florida, Gainesville, FL, USA, (2) North Carolina State University, Waynesville, NC, USA, (3) University of Central Florida, Orlando, FL, USA, (4) Connecticut Agric Exp Station, New Haven, CT, USA, (5) University of Florida, Quincy, FL, USA, (6) Department of Statistics, University of Florida, Gainesville, FL, USA, (7) Gulf Coast Research and Education Center, University of Florida, Wimauma, FL, USA, (8) Department of Plant Pathology, University of Florida, Gainesville, FL, USA

Bacterial spot of tomato, incited by four *Xanthomonas* species, is one of the most devastating bacterial disease in Florida. Since Copper (Cu)-tolerant strains rendered Cu bactericides ineffective, there has been no effective chemical control strategy. In 2017, the EPA has suggested that Cu bactericides be reduced on crops due to ecological risks. Therefore, finding an efficient alternative is an urgent need. Our research led to the discovery of novel antibacterial properties of magnesium (Mg) nano-materials against Cu-tolerant *X. perforans*. We demonstrate that a non-formulated magnesium oxide nanoparticles (Nano-MgO) have high bactericidal activity against *X. perforans*. Structural changes in the bacterial membrane following exposure Nano-MgO treatments were observed using transmission electron microscopy (TEM). In greenhouse and field experiments, disease severity was significantly reduced by Nano-MgO applications at 200 µg/mL compared to the untreated control (UT), whereas the grower standard treatment, copper-mancozeb (K+M), was not significantly different from UT ($p = 0.05$). In addition, fruits from Nano-MgO treated plots did not significantly alter levels of Mg, Cu, Ca, K, Mn, P and S compared to fruit in the UT. In current study, newly formulated Mg nano-materials (SgMc) with enhanced solubility significantly reduced disease severity to as low as 100 µg/mL compared to Cu+M and UT ($p = 0.05$) in two greenhouse studies. Plots treated with 100 µg/mL SgMc also showed significant disease reduction compared to Cu bactericide treated plots in the field. Based on the preliminary findings, we hypothesize that Mg nano-material will be an effective alternative to the current grower standard.

Dual transcriptomic analysis of the anthracnose fungal pathogen *Colletotrichum gloesporioides* infecting avocado

Chang Liu^{1,2,3}, Qiaolin Zheng¹, Prashant Hosmani⁴, Surya Saha⁴, Lukas Mueller⁴, Youjian Lin¹, Jiehua Zhu², Jiang Yin³, Brian Scully⁵, Patricia Manosalva⁶ and Liliana M. Cano¹, (1) University of Florida, Fort Pierce, FL, USA, (2) Hebei Agricultural University, Baoding, CHINA, (3) Hebei North University, Zhangjiakou, CHINA, (4) Boyce Thompson Institute, Ithaca, NY, USA, (5) USDA-ARS, Fort Pierce, FL, USA, (6) University of California, Riverside, CA, USA

Colletotrichum spp., are worldwide fungal plant pathogens that affect many economical crops. *Colletotrichum gloesporioides* (Cglo) is the causal agent of anthracnose on fruits and leaves of avocado (*Persea Americana* Miller). Anthracnose results in severe postharvest losses close to 20% of the total US production. The molecular basis of pathogenicity of this pathogen in avocado is largely unknown. To gain knowledge of the molecular interaction between this fungus and avocado, we conducted a dual RNAseq transcriptome study to identify key pathogen effector genes that are differentially expressed during infection in avocado fruits and leaves. We isolated eight field Cglo strains from fruit samples that exhibited anthracnose symptoms. The strain Cglo_Cg03 was used to infect detached fruits and leaves of 'Monroe' avocado cultivar. We evaluated the incidence of infected fruits and leaves at 7 days after inoculation which resulted in 100% and 96.5%, respectively. We collected both fruit and leaf infected tissues for RNA extraction and RNA-sequencing. We will be performing gene expression analyses using the publicly available genome of the avocado isolates *C. gloesporioides* strain CGLO01.

Impact of plastic mulch and tomato cultivar on disease incidence of tomato chlorotic spot orthotospovirus (TCSV)

Qingchun Liu¹, Bindu Poudel¹, Qingren Wang² and Shouan Zhang¹, (1) University of Florida, Homestead, FL, USA, (2) Miami-Dade County Extension, Homestead, FL, USA

Tomato chlorotic spot orthotospovirus (TCSV), transmitted by thrips, was first detected in 2012 from tomato and pepper in south Florida. Outbreaks have occurred since 2014, causing significant economic losses to tomato growers in this region. However, little information is available regarding TCSV epidemiology and its management. In November 2018, TCSV incidence was surveyed in three tomato fields in Homestead, FL., where two cultivars were planted on raised beds covered with two types of plastic mulches. Infection of both cultivars by TCSV was confirmed by a specific RT-PCR assay. TCSV incidence in field 1 on susceptible cv. 'Sanibel', planted 4 weeks ago, was the highest at the southeast corner (63.3% and 14.8%), and decreased northward (41.3% and 7.5% at the northeast corner) on white-on-black and UV-reflective plastic mulches, respectively. TCSV incidence was 29% and 11.0% at southwest corner and 11.3% and 0.5% at northwest corner on those two plastic mulches, respectively. Similar trend but lower TCSV incidence on both types of plastic mulches was observed on cv. 'Sanibel' planted 6 weeks ago in field 2, north next to field 1. In field 3, east next to field 1, TCSV incidence was 0.4% and 0.3% on those two plastic mulches, respectively, on resistant cv. 'Red Bounty' planted 3 weeks ago. The results from this survey indicated that the resistant cv. 'Red Bounty' and UV-reflective plastic mulch significantly reduced TCSV incidence in tomato fields in south Florida.

Colletotrichum spp. causing fruit rots in Kentucky: Investigating potential for cross-infection and developing improved tools for identification

Madison McCulloch, Etta M. Nuckles, Lisa J. Vaillancourt and Nicole A. Ward-Gauthier, University of Kentucky, Lexington, KY, USA

Fungi in the genus *Colletotrichum* cause apple, strawberry, and blueberry fruit rots, which result in significant losses for Kentucky growers. As mixed-fruit orchards become more common, the potential for cross-infection has management implications. Our first objective was to characterize *Colletotrichum* strains from different fruits in Kentucky orchards and determine host specificity. Multigene sequence analysis revealed that *C. nymphaeae* was most frequently found on strawberry, while *C. fioriniae* was the only species on blueberry. Both were previously reported on apple. We performed fruit inoculations with a representative group of isolates. Regardless of original host, all isolates caused disease on all three fruits. Accurate species identification is critical for management because *Colletotrichum* spp. differ in fungicide sensitivity, but sequencing methods are too slow for routine diagnosis. Our second objective was to evaluate sequences commonly used for multigene identification to determine if a single gene could accurately identify

the species. Multiple isolates were identified by standard multigene sequencing, and whole genomes of a representative group of isolates were compared to individual gene trees. This analysis revealed that the TUB2 sequence alone could accurately identify species. These findings will help us to develop more effective diagnostic and management procedures for *Colletotrichum* diseases in Kentucky mixed-fruit orchards.

Effect of New Non-fumigant Nematicides on Different Trophic Groups of Nematodes

David Moreira and Johan Desaegeer, University of Florida - Gulf Coast Research and Education Center, Wimauma, FL, USA

Plant parasitic nematodes cause more than \$100 billion dollars per year in damage to crops (>14% global crop production). Non-parasitic, free-living nematodes are critical components of the soil food web and play important roles in nutrient cycling and making inorganic chemicals available to plants. Management of plant parasitic nematodes has traditionally relied on the use of soil fumigants, and certain organophosphate and carbamate insecticides. Recently, new selective contact nematicides have emerged that offer alternatives to these restricted use pesticides. The effect on mortality and locomotion of varying concentrations of three new nematicides on different trophic groups of nematodes (bacterivore, fungivore and entomopathogenic) was tested *in-vitro*. Using 48-well tissue culture plates, nematodes were exposed to varying concentrations of the products and evaluated at five different time points (24h, 48h, 72h, 96h and 168h). Living and dead nematodes were determined by adding 1 N NaOH to each well and counting the nematodes that were able to react (alive) and the nematodes that were not able to respond (dead) to 1 N NaOH. Nematode mobility through soil was evaluated by placing nematodes on modified Baermann funnels following exposure to varying concentrations of the products (two time points, 12h and 24h). Results indicated that treatment effects are greater for fungivore and bacterivore nematodes than for entomopathogenic nematodes, especially at concentrations expected under field conditions. Nematode mobility is increasingly reduced at higher concentrations and after longer exposure to the products.

Detection of *Cercosporidium personatum* resistance to fungicides in South Carolina peanut fields

Misbakhul Munir¹, Hehe Wang¹, Paula A. Agudelo² and Daniel J. Anco¹, (1) Clemson University, Blackville, SC, USA, (2) Clemson University, Plant and Environmental Sciences Department, Clemson, SC, USA

Late leaf spot (LLS), caused by *Cercosporidium personatum*, is the most damaging fungal foliar disease of peanut in S.C. Control of LLS typically relies, in part, on repeated applications of fungicide. The occurrence of fungicide resistance by *C. personatum* population in S.C. was examined. In 2018, 72 groups of symptomatic leaves were collected from 9 counties. Per county, leaf samples were taken from 4 - 10 different fields. Spore suspensions (10⁵ spores/ml) obtained from each of the 72 groups were used to inoculate detached and fungicide-treated leaves to examine their sensitivity to 5 fungicides. Sensitivities were grouped into 4 categories (highly sensitive, moderately sensitive, moderately resistant, and highly resistant), based on ability to cause symptoms. Although all the 72 spore suspensions were highly sensitive to chlorothalonil, 32%, 26%, 25%, and 21% were highly or moderately resistant to azoxystrobin, thiophanate-methyl, prothioconazole, and benzovindiflupyr, respectively. Nine spore suspensions with resistance to at least 2 fungicides were observed. Except for chlorothalonil and control treatments, percentage symptom development in each fungicide treatment significantly varied by county ($P < 0.05$). These findings emphasize the importance of integrated pest management practices for LLS and resistance management in S.C. fields, as well as the utility of chlorothalonil tank mixes for potential combinations of location and risk of resistance to fungicides.

Comparison of RNA extracts from two extraction methods for *Rose rosette virus* detection

Katherine Olive, Madalyn Kirsten Shires and Kevin Ong, Texas A&M University, College Station, TX, USA

Efficient extraction of RNA is critical for downstream applications such as PCR detection assays. Commercially available extraction kits are effective, but costly. Some other methods require more time and are not as practical for diagnostic clinic operations. A Direct Antigen (DA) method was published in 2017 by Babu et al. and was optimized for use at the Texas Plant Disease Diagnostic Lab (TPDDL) for detection of *Rose rosette virus* (RRV). In this study, RNA extracted from this DA method was compared with extract from the standard Qiagen RNeasy[®] kit for stability and effects on detection sensitivity. RNA extracts from both methods were subjected to various simulated scenarios to mimic improper storage and handling, excessive freeze/thaw cycles, and errors in the DA extraction process. In a number of our experiments, the RNA from either extraction method appear to be stable and RRV was still detectable. There was a difference in the detection result in one scenario: 72 hours exposure of the RNA extracts at room temperature. Additional experiments will be performed to evaluate the impact of these simulated scenarios on samples with lower titers of RRV. This DA extraction method can be a useful, more rapid, more efficient and inexpensive alternative to the RNA extraction kits for diagnostic clinic applications.

Reduced aflatoxin contamination in transgenic maize lines containing a HIGS construct targeting *Aspergillus flavus* alkaline protease gene

Olanike Omotola Omolehin¹, Yenjit Raruang¹, Qijian Wei², Kanniah Rajasekaran², Deepak Bhatnagar² and Zhi-Yuan Chen¹, (1) Department of Plant Pathology and Crop Physiology, Louisiana State Univ Agric Center, Baton Rouge, LA, USA, (2) USDA-ARS, SRRC, New Orleans, LA, USA

Maize (*Zea mays*) is susceptible to *Aspergillus flavus* infection and subsequent aflatoxin contamination. Previous study demonstrated that *A. flavus* alkaline protease played a key role in the infection of maize kernels. Based on these findings, our objective was to investigate whether host induced gene silencing (HIGS) of an *A. flavus* alkaline protease (*ALK*) gene would result in reduced aflatoxin accumulation in maize. Transgenic seeds expressing a portion of *ALK* gene in B104 background were produced in 2013 and have gone through four rounds of self-pollination to produce homozygous transgenic seeds to evaluate their changes in aflatoxin resistance. Following field-inoculation at 14 days after pollination, the resulting T₄ lines from two transformation events were evaluated for aflatoxin accumulation. Up to 4-fold reduction in aflatoxin accumulation was observed in kernels of homozygous *ALK* lines compared to null and B104 controls ($P > 0.003$). To further test stability and heritability of the *ALK* transgene, T₄ homozygous *ALK* lines were crossed with elite inbred lines (PHN46 and LH195) and the F₁ kernels were inoculated under field condition. Compared to the elite line controls, the F₁ kernels accumulated 3-fold less aflatoxin ($P > 0.008$). Our results demonstrate the potential of enhancing maize resistance to aflatoxin accumulation by suppressing the expression of *A. flavus* alkaline protease gene through HIGS.

Secretome annotation of the citrus stem end rot fungal pathogen *Diplodia natalensis*

Egem Ozbudak¹, Guohong Liu^{1,2}, Qiaolin Zheng¹, Youjian Lin¹, Bo Liu², Prashant Hosmani³, Surya Saha³, Lukas Mueller³, Mark A. Ritenour¹ and Liliana M. Cano¹, (1) University of Florida, Fort Pierce, FL, USA, (2) Fujian Academy of Agricultural Sciences, Fuzhou, CHINA, (3) Boyce Thompson Institute, Ithaca, NY, USA

Diplodia species are fungal plant pathogens that are widely distributed in tropical and subtropical regions and cause different diseases in citrus. The control of *Diplodia* fungal infection in the field may reduce HLB-associated pre-harvest fruit drop. The type species *Diplodia natalensis* is synonym with *Lasiodiplodia theobromae*, *Botryodiplodia theobromae* and *Botryosphaeria rhodina*. Infection by *Diplodia* pathogens often results in soft brown rot of fruits shortly before or after harvest, and the symptoms are known as stem end rot (SER). In order to effectively control the spread of citrus *Diplodia* pathogens, it is necessary to identify their population. To present there are couple of specific primers to differentiate *Diplodia*, we have utilized EF1- α gene specific primer for *Diplodia* species for 13 isolates collected from South Florida between 2015–2017 and 2016. In this study we sequenced and assembled the genome for the strain Dnat_CITRA15 using PacBio which resulted in 475 contigs with a N50 of 178kb and with the sum of contigs equivalent to 43.3Mb. We have identified using Signalp software a set of 1,203 secreted proteins out of 10,456 predicted proteins in the genome. The long term goal is to use genome sequences to design new specific primers to improve species identification and also to determine the candidate pathogen disease related effector genes that could be used for functional analyses in future.

Gene expression analysis of *Colletotrichum acutatum* during infection in strawberry

Egem Ozbudak¹, Dorien Oppelaar¹, Marco Pitino¹, Prashant Hosmani², Natalia Peres³ and Liliana M. Cano¹, (1) University of Florida, Fort Pierce, FL, USA, (2) Boyce Thompson Institute, Ithaca, NY, USA, (3) Gulf Coast Research and Education Center; University of Florida, Wimauma, FL, USA

Colletotrichum acutatum, the causative agent of anthracnose crown and fruit rot, is recognized as the second most important pathogen of strawberries in the Globe due to its economic impacts. The disease causes characteristic sunken lesions with dark color on the strawberry fruits and blighted flowers. These symptoms making the fruits unsaleable in the markets. In order to protect the plants, the best way is to exclusion of the pathogen. Effectors are small secreted proteins released by pathogens for wide range of functions, such as invading the tissues and suppressing the immune system of host or increasing the survival of pathogen. Their recognition by resistance (*R*) genes elicits Effector-Triggered Immunity ETI and is associated with Hypersensitive Response. Therefore, presence of the *R* genes against effectors can be a matter of life or death during the infection. In this project, we performed a transcriptome assembly for three isolates of *C. acutatum* and the genes encoding for effectors proteins associated with anthracnose crown/fruit rot disease were determined. Currently, we are using a detached leaf assay to access the aggressiveness of different *C. acutatum* isolates infecting newly released strawberry cultivars. In addition, RNA will be extracted from infected leaf samples for RNA sequencing to select candidate effector genes expressed during infection. Our long-term goal is deploying these candidate effectors as molecular tools to fetch resistance genes in strawberry.

Evaluation of current and future methods of the National Cottonseed Treatment Trial

Shelly Pate, Heather M. Kelly and Rachel Guyer, University of Tennessee, Jackson, TN, USA

Over the past 23 years, the National Cottonseed Treatment Program has provided data on soil borne pathogens of cotton seedlings in effort to analyze seed treatment efficacy. A range of 12-20 cooperators have participated each year from universities in California, Texas, Oklahoma, Arkansas, Mississippi, Louisiana, Alabama, Tennessee, Virginia, South Carolina, Georgia, and Florida. Seeds treatments were nominated annually by chemical industry representatives and trials at each location analyzed their effects on cotton seedling survival over various environmental conditions and populations of four pathogens: *Pythium*, *Rhizoctonia*, *Thielaviopsis*, and *Fusarium*. Seedlings were evaluated for growth (node count), rated for disease severity, and plated on selective media for qualitative and quantitative analysis of each pathogen. In 2018, investigators at the University of Tennessee inherited the coordination of the NCST program and continued all protocols as they had been conducted during its 23-year history. With advancements in seed treatments and molecular technologies, the future methods used in this trial could be modified in an effort to provide more accurate data. This poster will discuss current materials and methods utilized for this trial, and how changes in future materials and methods will improve results.

Gene expression analysis of periwinkle infected with *Candidatus Liberibacter asiaticus*, the causal agent of Huanglongbing disease in citrus

Qiaolin Zheng¹, Marco Pitino¹, Yongping Duan² and Liliana M. Cano¹, (1) University of Florida, Fort Pierce, FL, USA, (2) USDA-ARS, Fort Pierce, FL, USA

Citrus greening also known as Huanglongbing (HLB) is one of most deadly citrus disease in the world. The causal agent is the fastidious bacterium *Candidatus Liberibacter asiaticus* (CLAs). CLAs can infect periwinkle *Catharanthus roseus* via grafting or dodder plants. Infected periwinkle exhibits greening-like symptoms, and it is recognized as a model system to study molecular basis for CLAs infection and host defense. Here, we used dodder to infect periwinkle with CLAs and collected tissues at different time points during infection for RNA sequencing. We used the publicly available gene calls sequences of periwinkle as reference to gene expression profiling of receptor and other defense related genes. Our long-term goal is to detect broad spectrum host susceptibility factors by comparing the differentially expressed genes found in periwinkle with citrus homolog genes previously reported to be altered during CLAs infection.

First report of *Fusarium fujikuroi* species complex causing leaf spot on Ohio spiderwort (*Tradescantia ohiensis*)

Sarah Prentice, Rosanne Healy and Brantlee Spakes Richter, University of Florida, Gainesville, FL, USA

Ohio spiderwort (*Tradescantia ohiensis* Raf., Commelinaceae) is a native perennial wildflower found throughout the eastern United States. In August 2017, chlorosis and necrotic spots were observed on leaves of Ohio spiderwort at the University of Florida Natural Area Teaching Lab, Gainesville, Florida. We consistently isolated a single fungus from symptomatic plants and confirmed pathogenicity through three repetitions of Koch's postulates. The fungus was identified as a *Fusarium* species based on colony and microscopic morphology. We extracted DNA, amplified the ITS region using the primers ITS1 and ITS4, and compared the sequence with GenBank accessions using BLAST. Results were a 100% match in identity and coverage to three species in the *Fusarium fujikuroi* species complex, fungi that are known pathogens of maize, rice, sorghum, and millet. Sequencing efforts are currently underway to refine the identification of this species. This finding demonstrates that a pathogen residing in a native plant population is closely related to crop pathogens, and Ohio spiderwort may serve as a reservoir for species pathogenic on crop plants. This is the first report of a *Fusarium* species causing disease on a *Tradescantia* species.

Determining the sensitivity of *Xylaria* sp. to commercially available fungicide active ingredients

Hope Renfro, Starkville, MS, USA, Tessie Wilkerson, Mississippi State University, Stoneville, MS, USA, Maria Tomaso-Peterson, Mississippi State University, Mississippi State, MS, USA and Thomas W. Allen Jr., MS, USA

Taproot decline (TRD) is a novel disease of soybean caused by an undescribed species of *Xylaria*. The primary diagnostic feature of TRD is black stroma on the tap and lateral roots. For this study, five *Xylaria* sp. isolates from Mississippi soybean were evaluated for sensitivity to thiophanate-methyl (TM), pyraclostrobin (PC), fluxapyroxad (FX), flutriafol (FT), and prothioconazole (PT). A stock solution of 1×10^4 µg/ml of each active ingredient was diluted to achieve concentrations between 0.0001 and 100.0 µg/ml. A 6 mm hyphal plug of each *Xylaria* sp. was transferred to fungicide-amended potato dextrose agar and incubated in the dark at 25°C for 10 days. Relative growth was calculated by dividing average radial growth by the average radial growth for the non-amended control and multiplied by 100. The effective concentration (EC₅₀), the dose required to inhibit colony growth by 50%, was calculated using PROC REG in SAS. All *Xylaria* sp. isolates were sensitive to TM with EC₅₀ values between 0.15 and 0.37 µg/ml. Intermediate sensitivity (EC₅₀ = 5.52 to 14.10 µg/ml) was observed when isolates were exposed to PC. A lack of inhibition was observed when isolates were exposed to PT indicated by EC₅₀ values between 28.83 and 40.58 µg/ml. A total lack of inhibition was observed at 100 µg/ml when isolates were exposed to FX and FT. Additional research is warranted to determine whether fungicide sensitivity or lack thereof translates to efficacy of these fungicides in the field.

A temporal analysis of the incidence of *Prune dwarf virus* and *Prunus necrotic ringspot virus* in stone fruit orchards in Texas

S. Corinne Rhodes, Texas A&M AgriLife Extension Service, College Station, TX, USA and Kevin Ong, Texas A&M University, College Station, TX, USA

Since 2011, the Texas Plant Disease Diagnostic Lab has participated in the National Stone Fruit Survey, monitoring for pests and pathogens of concern. At the request of the Texas Department of Agriculture, *Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV) were added to the survey to gain a better understanding of virus incidence and prevalence in Texas. Nine commercial peach orchards in nine different counties in Texas were tested for the presence of PDV and PNRSV. For orchards containing 200 trees or more, field samples were collected following a hierarchical sampling method, developed by Hughes and Gottwald (1999). For orchards containing more than 10 and up to 200 trees, an alternate sampling method was employed in which 100% of trees were sampled. For virus detection, each sample was tested by ELISA using commercially available ELISA kits from Agdia. Five orchards were tested in 2013, four orchards were tested in 2014, and all nine orchards were re-tested in 2018. For 2013 and 2014, 628 foliar peach, plum, cherry, and apricot samples were collected and tested. Fifteen samples tested positive for PDV and sixty samples tested positive for PNRSV. The surveyed orchards were revisited in 2018; 274 foliar peach and plum samples were collected and tested. Four samples tested positive for PDV and ten samples tested positive for PNRSV. Variability in viruses detected were noted in six counties over the sampling interval. However, impact of these infections are not known or not immediately noticed by the growers.

Occurrence and investigation of peach skin streaking in South Carolina peach orchards

Linus Schmitz and Guido Schnabel, Clemson University, Clemson, SC, USA

Club-shaped streaks lacking pigmentation have impacted red blush varieties of peaches in many east coast production areas, but the underlying causes are still unclear. The objective of this project was to monitor streaking occurrence and investigate potential causes by evaluating correlations to rainwater and reproducing symptoms in the lab. In 2017 and 2018, we monitored peach skin streaking at a commercial orchard in South Carolina, and Clemson Musser Fruit Research Center (CMFRC). Incidence of streaking varied depending on cultivar and location, ranging from 6% to 64%. Distribution of streaking within the canopy also differed based on location, with fruit from the top and outside areas of the canopy being predominantly affected in commercial orchards, while symptomatic fruit were distributed uniformly within the canopy at the CMFRC. The pH, electrical conductivity (EC), oxidation-reduction potential (ORP), and chlorine species present in rainwater were quantified. The pH values ranged from 3.03 to 7.4, while EC values were below $100 \mu\text{S cm}^{-1}$ on average, indicating low ionic strength. For all samples the ORP indicated oxidizing conditions. Chlorine levels were under the detection limit of 0.01 mg L^{-1} for Cl_2 and 0.02 mg L^{-1} for ClO_2 . Inoculation of detached fruit with solutions of high (10) or low (3) pH and EC values above $100 \mu\text{S cm}^{-1}$ did not result in streaking, whereas high ClO_2 levels (500 mg L^{-1}) did. Other hypotheses for potential causes are being evaluated.