2020 Southern Division Meeting Abstracts

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Control of *Phytophthora* root rot of boxwood with chemical fungicides, biofungicides, fertilizers and host plant defense inducers in field production

S. NEUPANE, T. Simmons, F. Baysal-Gurel, Tennessee State University, McMinnville, TN, USA

Phytophthora root rot, caused by *Phytophthora nicotianae*, can affect all growth stages of field grown boxwood plants. Management is a problem and is possible only through an integrated approach. In this study, we evaluated the efficacy of fungicides, biofungicides, fertilizers, and host plant defense inducers to control *Phytophthora* root rot of boxwood. Field experiments were conducted in 2018 and 2019. Plots were inoculated with *P. nicotianae* grown on rice grains. Plant height and fresh weight were recorded, and roots were assessed for disease severity using a scale of 0–100% roots affected. The treatments most effective in reducing *Phytophthora* disease severity were Orkestra Intrinsic, Subdue MAXX, Segovis, fertilizer ON-Gard alone and the RootShield PLUS⁺ + fertilizer ON-Gard application program. Disease severity for those treatments were not significantly different than the non-treated, non-inoculated control in 2018. All of the treatments except Subdue MAXX and Aliette resulted in a significantly greater plant height compared to the non-treated, inoculated control in 2018. There were no significant differences in disease severity between treated, with the exclusion of Areca, and non-treated, non-inoculated control in 2019. There were also no significant differences in plant height and fresh weight between treated and non-treated control boxwood plants in 2019.

Sensitivity of *Corynespora cassiicola* from cotton and soybean to DMI and QoI fungicides

M. N. RONDON (1), K. S. Lawrence (2), (1) Auburn University, Auburn University, AL, USA; (2) Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

Corynespora cassiicola, the causal agent of target spot, is a fungal pathogen with increasing occurrence causing significant yield losses in cotton and soybean. Fungicides are a crucial tool in disease management; although, different resistance levels of *C. cassiicola* to several fungicide groups have been reported, including MBC, SDHI and QoI. Our objective was to determine the effective fungicide concentration at which 50% of mycelial growth was inhibited (EC₅₀) with prothioconazole (DMI) and pyraclostrobin (QoI) fungicides on cotton and soybean isolates of *C. cassiicola* from Alabama. EC₅₀ of *C. cassiicola* isolates to the fungicides was tested using an in vitro mycelial growth assay in amended media. EC₅₀ values were estimated by nonlinear regression using PROC NLIN in SAS 9.4. EC₅₀ values of prothioconazole ranged between 0.32–1.43 mg/L for cotton isolates and ranged between 0.29–2.35 mg/L for soybean isolates. EC₅₀ values of pyraclostrobin ranged between 3.39–13.77 mg/L for cotton isolates and ranged between 9.59–72.12 mg/L for soybean isolates. The results suggested that soybean isolates have been exposed to these fungicides more often than cotton isolates. EC₅₀ values of pyraclostrobin above 46 mg/L were found for isolates from soybean only, indicating loss of sensitivity of *C. cassiicola* isolates to the active ingredient. Our results will be useful to monitor resistance to fungicides of *C. cassiicola* and help with fungicide resistance management.

Advanced molecular methods for detection and race differentiation of *Fusarium oxysporum* f. sp. *niveum*

O. HUDSON (1), S. Waliullah (2), P. Ji (1), E. Ali (1), (1) University of Georgia, Tifton, GA, USA; (2) University of Georgia, GA, USA

Fusarium oxysporum f. sp. *niveum* (FON) is the causal agent of Fusarium wilt, a devastating fungal disease that limits production of watermelon internationally. Four races of FON (0, 1, 2, and 3) have been identified, with race 3 being the most aggressive and having no resistant watermelon cultivars. Differentiation of FON races currently relies on inoculation of a set of differential watermelon cultivars/accessions and evaluation of disease development. This method is time consuming, labor intensive, and often produces inconsistent results due to changes of environmental conditions and subjective scoring of disease intensity. Molecular detection methods for differentiation of FON races was not available. In this study, we developed a new Loop Mediated Isothermal Amplification (LAMP) assay for specific detection of FON which could detect a level of DNA as low as 0.01 pg of genomic DNA and did not amplify the closest related pathogenic formae speciales: *Fusarium oxysporum* f. sp. *lycopersici*. We also present a novel LAMP assay for the differentiation of race 2 from the other races of FON. Additionally, we have done a whole genome sequencing of multiple races and have found significant differences among races. We report our progress here to develop candidate markers for differentiation of other FON races. Our findings will help growers immensely due to the decreased amount of time and resources previously dedicated to determining the pathogen's race.

Evaluation of laser-guided air-blast sprayer on pest and disease control in peach orchards

H. BOATWRIGHT, G. Schnabel, Clemson University, Clemson, SC, USA

Commercial peach production relies on aerial application of pesticides to control pests and disease. Air-blast sprayers catapult chemicals into the tree row without distinguishing trees, tree gaps, or canopy density. To reduce amount of spray materials used and lost to off-target sprays and drift, the Ohio University and the USDA-ARS developed an Intelligent Sprayer (iSprayer) system that can be retrofitted onto existing sprayers. The iSprayer consists of a laser scanning sensor, solenoid assisted nozzles, and an onboard computer system and costs approximately \$27,000 plus installation fees. A Durand Wayland air-blast sprayer was fitted with the iSprayer and used in cultivars 'Juneprince' and 'PF23' over the 2019 season to determine pest and disease control efficacy. Field trials showed the iSprayer as effective as a conventional air-blast sprayer in controlling preharvest and postharvest brown rot and catfacing despite significant reductions in spray volume. Younger trees had a 55%–71% reduction in spray material used whereas older trees only had a 13%–50% reduction throughout the season. The highest percent reduction in material occurred in the early season. Drift was reduced with the iSprayer while maintaining comparable coverage of the canopy. In conclusion, the iSprayer retrofit appears to significantly reduce spray volume and drift while maintaining disease efficacy, but if the iSprayer is cost effective needs to be investigated.

Survey of soil and foliar fungal pathogens observed in watermelon production fields throughout the growing regions of Texas

B. MEYER (1,2), K. A. Cochran (3), K. Ong (1), (1) Texas A&M AgriLife Extension Service, College Station, TX, USA; (2) Texas A&M University, College Station, TX, USA; (3) Texas A&M University, Uvalde, TX, USA

Texas Watermelon production ranks first in the nation with average revenue of \$8 million. Fungal pathogens such as *Fusarium spp.* can cause millions in lost revenue if not controlled. Environmental conditions such as precipitation, wind speed/direction, and temperatures (high and low) can vary throughout Texas and influence species compositions. This survey was conducted to document fungal pathogens present during the 2019 growing season and lead to understanding Texas production challenges. Seven commercial watermelon fields in five Texas counties were sampled for this survey. A random sampling method was used with 50 samples taken from fields with larger than 50 acres and 30 samples from fields smaller than 50 acres; GPS coordinates of field position were taken for each sample for disease mapping. To perform fungal pathogen assays, stem and/or root samples were surface sterilized, plated on 25% PDA amended with antibiotics, and grown for one week at 27°C. Plates were observed for fungal culture growth and each fungal colony was identified using morphological traits. Five fungal pathogens were observed: *Fusarium oxysporum*, *Macrophomia phaeolina*, *Alternaria cucumerin*, *Colletotrichum orbiculare*, and *Rhizopus stolonifera*. Distribution of fungal species varied at different survey sites. This data can lead to a better understanding of pathogen community composition and prevalence leading to improved disease prediction and the development of better management practices.

Occurrence of the root-knot nematode *Meloidogyne enterolobii* infecting sweetpotato in North Carolina, United States

W. YE (1), T. Schwarz (2), E. L. Davis (3), L. D. Thiessen (3), L. Quesada-Ocampo (3), A. M. Gorny (2), (1) North Carolina Department of Agriculture & Consumer Services, Raleigh, NC, USA; (2) Department of Entomology and Plant Pathology, North Carolina State University, NC, USA; (3) North Carolina State University, Raleigh, NC, USA

Meloidogyne enterolobii is a recently detected and emerging root-knot nematode (RKN) species in the southeastern United States (US), causing severe damage to sweetpotato, soybean, and cotton. In November 2014, sweetpotato (cv. Covington) samples were collected from Johnston County, North Carolina. Fibrous and storage roots displayed significant galling damage, suggestive of RKN. Nematode extraction and quantification from accompanying soil samples indicated 1,000 to 4,200 second-stage juveniles per 500 cc soil. DNA was isolated by plucking and crushing individual female nematodes (n = 5) on a microscope slide. Sequencing of the 18S rDNA, internal transcribed spacer region 1, 28S rDNA D2-D3, and COII-16S rDNA was performed for species identification. BLAST searches in the GenBank database indicated sequencing results were identical to published DNA sequences of *M. enterolobii*. Koch's postulates were completed in the greenhouse. All inoculated plants displayed galling damage and no galling was observed on control plants. North

Carolina is ranked as the No. 1 sweetpotato producing state in the US. From sweetpotato, *M. enterolobii* has been confirmed in limited fields in Columbus, Craven, Greene, Harnett, Johnston, Lenoir, Nash, Pitt, Sampson, Wayne, and Wilson counties as of December 2019. *Meloidogyne enterolobii* is a major concern to sweetpotato growers in North Carolina and the southeastern US because it affects both yield and quality of sweetpotato.

Use of telomere fingerprinting to identify *Colletotrichum fioriniae* clones and application to cross-infection studies in Kentucky mixed-fruit orchards

M. MCCULLOCH, E. M. Nuckles, L. J. Vaillancourt, N. A. Ward-Gauthier, University of Kentucky, Lexington, KY, USA

Colletotrichum fruit rots can cause significant losses in apple, blueberry, and strawberry worldwide. Most orchards in Kentucky are focused on agritourism and typically grow different fruit crops in close proximity. These mixed-fruit orchards may facilitate cross-infection by *Colletotrichum* spp., which may have serious management implications. *Colletotrichum fioriniae* is the most prominent *Colletotrichum* species in Kentucky orchards. Our primary objective is to determine whether cross-infection occurs in these mixed-fruit orchards. Telomere fingerprints, identified by Southern blotting, were used to identify clonal strains and to assess cross-infection occurrence. Telomere fingerprinting of a diverse set of *C. fioriniae* isolates representing a broad host and geographical range revealed numerous polymorphisms, indicating that the method may be used to differentiate among clonal lineages. We deeply sampled *C. fioriniae* isolates from three Kentucky orchards with the goal of using the telomere fingerprinting method to determine whether individual clones were present on more than one fruit type within a single orchard. Identical fingerprints will indicate that cross-infection has occurred across fruit types. Understanding more about cross-infection and movement of *Colletotrichum* isolates in the field will help to inform and improve fruit anthracnose management practices.

Host-specific effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*

S. PURAYANNUR (1), L. M. Cano (1,2), M. Bowman (3), K. L. Childs (3), L. M. Quesada (1), (1) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA; (2) University of Florida, Institute of Food and Agricultural Sciences, IRREC, Fort Pierce, FL, USA; (3) Department of Plant Biology, Michigan State University, East Lansing, MI, USA

Downy mildew caused by the biotrophic oomycete *Pseudoperonospora cubensis* is one of the most devastating diseases affecting crops belonging to the Cucurbitaceae family. Several studies have noted the existence of two distinct host-specialized clades in *P. cubensis*, Clade 1 and Clade 2. The introduction of Clade 2 is thought to have caused the resurgence of cucumber downy mildew in 2004 in the US. In an attempt to identify the differential RXLR effectorome of Clade 1 and Clade 2, we sequenced the genome of one representative isolate of each clade. In-silico prediction led to the identification of ~1000 secreted proteins in both clades. Using pattern search and sequence similarity analysis in the R package effectR and a Galaxy toolshed designed for oomycete effectors, we discovered about ~200 genes encoding RXLR motif-containing effectors. Gene orthology analysis revealed the presence of 136 clusters common to both clades, and 41 and 27 clusters specific to Clades 1 and 2, respectively. RNA-seq analysis in the sporangia of selected Clade 1 and Clade 2 isolates showed contrasting transcript evidence for some effector-encoding genes. An RNA-seq time-course experiment revealed the expression pattern of the identified effectors at different stages of infection for both clades. We propose that the identified RXLR effector suite could be used towards effector-assisted breeding to accelerate the development of downy mildew-resistant cucurbits.

Phylogeography of *Ditylenchus gallaeformans*

S. AZEVEDO DE OLIVEIRA (1), P. A. Agudelo (2), S. DeWalt (3), (1) Greenville, SC, USA; (2) Clemson University, Plant and Environmental Sciences Department, Clemson, SC, USA; (3) Clemson University, SC, USA

Ditylenchus gallaeformans has been considered as a possible biological control agent against invasive plant species. However, the culture of this nematode has not been established yet. For this reason, it is important to investigate its biology and geographic distribution to understand its relation with the hosts and environment. Understanding *D. gallaeformans* phylogeography may provide insight into the coevolution with its host, phylogeny and genetic differences among populations, which may have a role on host preference, virulence and pathogenesis. In this study we used nematodes from Costa Rica, Dominica and Trinidad. DNA was extracted from individual nematodes and the COI region was amplified and sequenced. Phylogenetic analysis was performed using maximum-likelihood (ML) and Bayesian inference (BI), and haplotype networks were constructed according to geographical location and host species. The phylogenetic reconstruction yielded trees with similar topologies and revealed four major clades by location. Haplotype networks were congruent with the phylogenetic trees, showing segregation of haplotypes according to locality and not by host species. The results show us that our *D. gallaeformans* samples are monophyletic, and that the *Ditylenchus* group is paraphyletic. The haplotype networks revealed that geographic location is the trait responsible for the population structure observed, and the host species have no influence on the haplotypes.

Chemical management to reduce tomato spotted wilt virus on tobacco in North Carolina

Y. I. ROSADO RIVERA, H. Burrack, A. Toennissen, L. D. Thiessen, North Carolina State University, Raleigh, NC, USA

Tomato spotted wilt virus (TSWV) is a devastating disease of tobacco affecting several counties of North Carolina which is vectored by thrips. Currently, there are limited management practices to reduce yield losses caused by TSWV. Tobacco thrips management along

with timed applications of plant defense elicitors may decrease crop losses. Thus, the objective of this trial was to evaluate the effect of different chemical products prior to transplanting combined with the thrips flight model prediction on TSWV incidence. A field experiment comprised of seven treatments and four replicates organized in a randomized complete block design was conducted in 2018 and 2019 in Sampson County, North Carolina. Admire Pro (a.i. imidacloprid), Ninja (a.i. ningenmycin), and Actigard (a.i. acibenzolar-S-methyl) were evaluated at different rates and timings. Stand count, plant vigor, and disease incidence were assessed every two weeks beginning at transplant. Data was summarized using a weighted least squares model with a beta regression and Tukey's test for means separation. Differences in disease epidemics were observed between years, and disease incidence was higher in 2018 than 2019 ($p = 0.001$). In 2018, drench application of Admire Pro three days before transplanting followed by an application of Actigard three days before the peak of the third thrips flight significantly reduced the disease incidence when compared to other treatments ($p = 0.003$).

Phylogenetic informativeness of mitochondrial protein-coding genes in Nematoda

X. MA (1), J. A. Baeza (2), V. Richards (2), P. A. Agudelo (3), (1) Clemson University, Clemson, SC, USA; (2) Clemson University, SC, USA; (3) Clemson University, Plant and Environmental Sciences Department, Clemson, SC, USA

We investigated phylogenetic signals of 92 nematode mitochondrial genomes from 65 genera including a recently sequenced plant-parasitic nematode species (*Hoplolaimus columbus*) for better understanding of the utility of mitochondrial protein-coding genes (PCGs) for nematode phylogenetic studies. The investigations were initiated from generating phylogenetic trees using various patterns of PCG marker combos, including 43 Maximum Likelihood trees, 43 Bayesian Inference trees, and 1 Species Divergence Time tree. Two methods for phylogenetic signal analyses were used. The first method compared phylogenetic results with different mitochondrial markers by the Environment for Tree Exploration v3, calculating topological distances and similarities among trees. The second calculated and compared the phylogenetic informativeness values of those PCG markers on PhyDesign. Results from the two methods were in agreement and suggested that the phylogenetic signal embedded in a marker should be considered in order to reconstruct a reliable phylogenetic relationship. We also found that phylogenetic signal is gene-specific, and independent of the gene size. The *nad5* and *nad4* genes are highly informative for nematode phylogeny. Traditional mitochondrial markers, such as the *cox1* or *cytb* gene, contain only moderate phylogenetic informativeness. We do not recommend using *nad3*, *nad4L*, *cox2* or *nad6* gene for phylogenetic reconstruction given their low informativeness.

Fungicide sensitivity screening for *Corynespora cassiicola* and field evaluations

T. M. SMITH (1), H. M. Kelly (2), Z. R. Hansen (3), (1) University of Tennessee, Jackson, TN, USA; (2) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA; (3) University of Tennessee, Knoxville, TN, USA

Target spot (TS), caused by the fungus *Corynespora cassiicola*, is a foliar disease of over 530 plant species, with several being economically important crops. Over recent years, TS has become a disease of concern in both cotton and soybean production systems. Data for fungicide sensitivity and understanding potential impact on yield is lacking for *C. cassiicola*. The objective of this study is to monitor fungicide sensitivity in *C. cassiicola* in Tennessee soybean and cotton production. The sensitivity of 30 *C. cassiicola* isolates to 8 technical grade fungicides across multiple fungicide groups (FRAC Groups 1, 3, 7, and 11) was evaluated based on mycelial growth inhibition assays. The EC_{50} of each fungicide was calculated. Based on preliminary analysis there is variability in sensitivity to FRAC Groups 11 and 3. Fungicide field trials were also conducted at 3 locations for soybean and 1 location for cotton. Five fungicide tank mixes were evaluated for control of TS among 3 soybean varieties of differing susceptibility and 6 fungicide products were evaluated among 1 cotton cultivar. While all product mixes significantly reduced TS compared to the non-treated check, soybean yield did not significantly increase. No treatments were significantly different in the cotton trial. Additional details of fungicide sensitivity screening and field trial results will be presented.

Identification of accessions of melon (*Cucumis melo*) germplasm with resistance to bacterial wilt of cucurbits using mechanical inoculations

B. ACHARYA (1), L. Mackasmiel (2), K. Dumenyo (1), (1) Tennessee State University, Nashville, TN, USA; (2) Tennessee State University, Woodbury, TN, USA

One of the most devastating diseases of plants in the Cucurbitaceae family is bacterial wilt of cucurbits (BW), caused by the Gram-negative bacterium, *Erwinia tracheiphila* (Et). The pathogen is vectored by striped cucumber beetle (*Acalymma vittatum*) and spotted cucumber beetles (*Diabrotica undecimpunctata*). The highly susceptible species include melon (*Cucumis melo*) and cucumber (*Cucumis sativus*). There are no lasting resistance cultivars and no effective pathogen-based disease management strategy as infected plant ultimately die. Broad-spectrum insecticides have adverse effects on beneficial insects such as pollinator species. The purpose of this study was to screen melon accessions for resistance against bacteria wilt disease in a greenhouse trial in fall 2019. Plants of melon accessions obtained from USDA germplasm collection were mechanically inoculated with Et strain HCa1-5N with a modified inoculation method. We recorded the number of days to wilting of inoculated leaf (DWIL), days to wilting of whole plant (DWWP) and days to death of the plant (DDP). Two accession, PI 200814 and PI 230186 had significantly higher DWIL, DWWP and DDP while four lines (PI 344436, PI 618838, Ames 13299, PI 378060, PI 502328, PI 211016) were higher in DWWP and DDP. Nineteen other accessions had a significantly higher DWIL, DWWP or DDP. The genetic sources of these variations will be employed in melon breeding programs against BW.

Hemp leaf spot, a major disease threat for hemp, is caused by haploid and heteroploid populations

D. SZARKA, J. Jaromczyk, C. L. Schardl, N. A. Ward-Gauthier, University of Kentucky, Lexington, KY, USA

Hemp leaf spot emerged as a new disease on hemp in Kentucky in 2014, coinciding with the reintroduction of the crop. The disease has since been confirmed in at least 18 counties throughout Kentucky; similar cases have been reported from at least 15 states. *Drechslera gigantea* was confirmed as the causal agent of hemp leaf spot, and phylogenetic analysis indicated that they should all be reclassified in genus *Bipolaris*. Further, these populations were comprised of both heteroploid and haploid strains. Morphological and molecular comparisons were conducted on isolates from symptomatic fields from eight counties in Kentucky. Isolates were determined to be similar in appearance and morphology. Sequence analysis of several genes, as well as whole genome analyses, indicated that some isolates were haploid, but others contained two alleles of each gene, two different mating type genes, and genome sizes approximately double the size of haploid genomes. These apparent heteroploids were closely related to the haploids. Understanding the genetics and diversity of the causal pathogen is critical for developing and implementing effective management strategies. As hemp acreage continues to increase, the potential impact of this disease may cause significant yield loss that threaten the future of this new crop.

Identification and biocontrol potential of antagonistic bacterial strains against *Pseudomonas syringae* pv. *tomato*

C. OKSEL (1), F. Baysal-Gurel (1), M. Mirik (2), (1) Tennessee State University, McMinnville, TN, USA; (2) Namik Kemal Univ, Tekirdag, TURKEY

Bacterial speck of tomato, caused by *Pseudomonas syringae* pv. *tomato* (*Pst*), continues to be a problem for tomato growers worldwide. Biological control is achieved through the use of microorganisms to suppress the growth and development of plant pathogen and ultimately reduce the possibility of disease onset. In this study, soil samples were collected from healthy tomato fields and 127 strains were tested against *Pst* with a dual culture method for their antagonistic activity. Seven strains exhibited considerable inhibitory activity *in vitro* and they were identified as *Bacillus mycoides*, *Rhizobium radiobacter*, *P. corrugata*, *Alcaligenes faecalis*, *B. simplex*, *P. chlororaphis* and *B. pumilis* using MALDI-TOF MS. These bacteria were further tested on four-week-old tomato seedlings for their efficacy in reducing bacterial speck disease severity. Plants were sprayed with a 10⁸ CFU/mL cell suspension of bacterial strains. After 24 hr, the plants were inoculated with *Pst* strain RM-Ö-1-1r. The severity of bacterial speck was evaluated 21 days after inoculation using a scale of 0–100% foliage area affected. *B. simplex*, *A. faecalis* and *R. radiobacter* significantly reduced bacterial speck disease severity (47.2, 44.6 and 42.8%, respectively) compared to others and the non-treated control. Bacterial strains from this study may be considered as promising candidates to develop new biocontrol agents for controlling bacterial speck of tomato.

Palm diseases and their management

B. DHILLON, University of Florida, Davie, FL, USA

Palms are ‘the’ iconic plant species of the tropics. There are approximately 2,600 species of palms that dot the landscape in tropical and sub-tropical ecosystems. Palms are an important part of the social and economic spheres, both globally and locally. In 2016, the palm oil industry was responsible for 2.9 million jobs world-wide and contributed \$39 billion to the global GDP. Palm has its cohort of described fungal pathogens as well as newly emerging pathogens. Currently, three major groups of pathogens, *Fusarium*, *Ganoderma* and *Phytophthora*, are seen as critical threats for palm production. In order to better manage the diseases and associated losses, there is a need to characterize the palm ‘pathobiome’ across the landscape. The overarching goal of this project would be to establish projects that aim to 1) identify factors involved in host resistance and pathogen virulence; and 2) control and manage palm diseases using traditional, molecular and next-gen methodologies. This work will primarily focus on issues faced by stakeholders in Florida and the US, and at the same time build collaborative efforts across the globe. Using a proactive approach to understand palm diseases would enable us to address the biotic challenges faced by stakeholders at the landscape level as well as in the plantations.

An assessment of seed treatment efficacy and cotton seedling disease presence using innovative techniques

S. PATE (1), H. M. Kelly (2), R. Guyer (2), (1) University of Tennessee, Jackson, TN, USA; (2) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA

The goal of the National Cottonseed Treatment, NCST, program is to analyze seed treatment efficacy and stand persistence, in relation to pathogens present in the soil of cotton plots across the United States Cotton Belt. Through executing the existing protocol and analyzing the data collected from the program for the 2018 growing season, it was apparent that changes needed to be made in order to enhance the sampling process and make the data generated more accurate. Our updated protocol now includes plating seedlings from each field trial location on different selective medias for the morphological identification of *Thielaviopsis basicola*, *Pythium* spp., and *Fusarium* spp. As well as soil baiting for *Rhizoctonia solani*, and enzyme-linked immunosorbent assays (ELISA)s for *Pythium* spp. and *R. solani* detection. To further enhance *Pythium* data, isolates’ pathogenicity was evaluated using a seed germination assay. With more accurate data, we are able to better interpret seed treatment efficacy in relation to pathogens managed at each field site location and will use this to build risk models. In the presentation, data yielded from detection techniques will be compared outlining the most accurate, time efficient, and cost effective techniques and further utility of the data for modeling.

Steam-based thermotherapy for non-chemical management of nematodes in strawberry transplants

J. Desaeger (1), C. KHANAL (2), H. Regmi (1), (1) University of Florida, Wimauma, FL, USA; (2) University of Florida, Riverview, FL, USA

Strawberry transplants shipped to Florida from out-of-state nurseries have been found to harbor *Aphelenchoides besseyi*, *Meloidogyne hapla* and *Pratylenchus penetrans*. Studies were initially focused on evaluating the intrinsic temperature sensitivity of each nematode species. This was done by exposing nematodes for 1, 5, 10, 30, 60, 120 and 240 min at 40, 44, 48 and 52°C. Exposure for 60 min or higher at 40°C paralyzed all three nematode species when examined immediately after heat treatment. Similarly, minimum exposure times to paralyze all nematode species at 44, 48 and 52°C were 60, 10 and 5 min, respectively. Nematodes were re-evaluated after 24 h to evaluate if nematodes can recover from the initial heat stress. Overall, *M. hapla* was more sensitive to heat treatment followed by *A. besseyi* and *P. penetrans*. The minimum exposure time and temperature combinations required to achieve 100% mortality of all nematode species 24h post treatment were 120 min at 44°C, 10 min at 48°C, and 5 min at 52°C. We further conducted greenhouse studies to evaluate the efficacy of aerated steam at 44°C to effectively kill all three nematode species by exposing nematode-infested strawberry transplants for 1, 2 and 4 h. Exposure of strawberry transplants infested with *A. besseyi* to steam for 4 h effectively killed the nematode. Greenhouse studies are currently ongoing to determine efficacy of aerated steam to kill the other two nematode species in strawberry transplants.

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M. J. NEWARK (1), S. Ludwig (2), W. Carter (3), R. Rice (4), (1) AMVAC Chemical Corporation, Gainesville, FL, USA; (2) AMVAC, Arp, TX, USA; (3) AMVAC, Tifton, GA, USA; (4) AMVAC, Collierville, TN, USA

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Potential host range and effects of taproot decline on rotation cover crops

J. Moscoso (1), S. A. Winters (2), A. ROJAS (3), (1) Entomology and Plant Pathology, University of Arkansas - Division of Agriculture, Fayetteville, AR, USA; (2) Entomology and Plant Pathology, University of Arkansas - Division of Ag, Fayetteville, AR, USA; (3) University of Arkansas, Fayetteville, AR, USA

Taproot decline, caused by *Xylaria* cf. *arbuscula* is an emergent disease of soybean in the southern United States. The pathogen colonizes the root system causing necrotic symptoms on the taproot and continuous infection causes yellowing of the foliage that turns into necrotic lesions. *Xylaria* is a well-known genus of saprophytic fungi, but often members of this genus are also associated with the functional guild of endophytes. Taproot decline has recently gained attention and cultivar screening and seed treatments have been assessed as strategies to manage this disease. However, other cultural practices such as crop rotation and enhancement of soil health by using cover crops could have the potential on changing the environment where this disease occurs. The hypothesis is that cover crops could serve as potential hosts for this pathogen or the increase of organic matter will favor the prevalence of *Xylaria*. In order to test this hypothesis we adapted inoculation methods to study the potential of *Xylaria* to survive and colonize different cover crops used in Arkansas. The cover crops studied includes winter pea, radish, wheat, mustard and cereal rye. Two isolates were tested on seed plate assays and seedling assays. The responses varied among the cover crops, either reducing, not affecting or increasing biomass. The potential survival of *Xylaria* cf. *arbuscula* on cover crops will be useful in considering rotations for management of taproot decline.

Integrating beneficial bacteria for management of southern stem rot and late leaf spot in peanuts

A. A. HERBERT (1), D. J. Anco (2), H. Wang (3), (1) Clemson University, Clemson, SC, USA; (2) Clemson University, Blackville, SC, USA; (3) Clemson University - EREC, Blackville, SC, USA

Southern stem rot caused by *Sclerotium rolfsii*, and late leaf spot caused by *Nothopassalora personata*, are two major diseases in the South Carolina peanut production. Current disease management utilizes targeted chemical applications, however, in the presence of inoculum and favorable weather conditions, either disease can result in losses over \$ 250 / hectare. This study aimed to identify and evaluate the efficacy of beneficial bacteria to inhibit the growth of fungal pathogens and improve peanut yield. First, a field trial was conducted to compare the in-furrow and spray treatments of commercially available biocontrol products versus standard chemical treatments. Then, novel biocontrol agents were isolated from rhizobacteria populations across South Carolina peanut fields and screened in the lab and greenhouse against *S. rolfsii* and *N. personata*. In the 2019 field trial, all biocontrol and chemical treatments were shown to have comparable effects on controlling late leaf spot, and the higher peanut yield was observed in the biocontrol treatments. Disease pressure of stem rot was too low to show any difference among treatments. Of the 326 bacterial isolates screened, 14 *Bacillus* isolates showed significant antagonization of *S. rolfsii* growth *in vitro* and were further tested on inoculated plants in the greenhouse. This project showed the promising potential of beneficial bacteria in promoting peanut yield and managing stem rot and late leaf spot of peanut.

Utilizing a population genetics approach to provide crop-specific management strategies for cucurbit downy mildew

K. D'ARCANGELO (1), A. Rahman (2), T. D. Miles (3), L. M. Quesada (4), (1) North Carolina State University, Raleigh, NC, USA; (2) Corteva Agrisciences, Indianapolis, IN, USA; (3) Michigan State University, East Lansing, MI, USA; (4) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Following a population shift in *Pseudoperonospora cubensis* in 2004, host resistance was lost and a change in fungicide sensitivity was observed in the field, rendering many commercial fungicides ineffective. It was determined that two *P. cubensis* clusters representing host-specific clades were present using microsatellite markers (SSRs). To differentiate isolates, a clade-specific assay was developed using the nuclear genome. A follow-up study found that SNPs known to confer resistance to FRAC 40 fungicides were more frequently found in Clade 2. To further study this phenomenon, trials were conducted in 2016 and 2017 with the objective of determining the genetic structure of *P. cubensis* populations immediately following single-product fungicide applications on cucumber (Clade 2 isolates). Using SSRs, a clade-specific assay, and TaqMan qPCR assays to detect FRAC 11 and 40 fungicide resistance SNPs, 880 *P. cubensis* isolates were genotyped. A STRUCTURE analysis revealed distinct genetic shifts following fungicide applications and similar clusters were present in non-treated varieties with the same sources of host resistance. These results provide valuable insight into the role fungicides and host tolerance play in the evolution of *P. cubensis* populations and highlight the need for crop-specific management strategies to more reliably control cucurbit downy mildew.

Micronutrients affect induced resistance in watermelon against *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita* infection

K. KARKI (1), B. Dutta (1), A. Hajiassani (1), T. W. Coolong (1), C. S. Kousik (2), M. K. Mandal PhD (3), (1) University of Georgia, Tifton, GA, USA; (2) U.S. Vegetable Laboratory, USDA ARS, Charleston, SC, USA; (3) Claflin University, Orangeburg, SC, USA

Fusarium oxysporum f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*) are devastating soil-borne pathogens of watermelon in the southeast United States. It is hypothesized that soil micronutrients may play an important role in disease severity; however, information is lacking in this aspect. We utilized a hydroponics system to investigate if Mn, Zn, and Fe treatments can differentially affect induced resistance (IR) genes [SA pathway (PR1, NPR1, PR5) and JA pathway (LOX, VSP, and PDF)] in watermelon seedlings prior and post-pathogen inoculation (FON, RKN or FON+RKN). Micronutrients were fed to the plants at higher (3X) or lower (0.5X) levels than the standard dose (X) for 7 days and the expression levels of IR genes were evaluated. A sub-set of micronutrient-fed plants was later inoculated with either FON, RKN or both, and re-evaluated at 3-day post-inoculation for IR genes. Our results showed that expressions of defense-related genes were not activated pre-inoculation. However, upon pathogen inoculation (FON, RKN or both), both SA and JA genes were activated and expression levels varied considerably for different micronutrient treatments. These observations indicate that micronutrient concentrations in growth-substrate and plant tissues can affect genes in SA and JA pathways during FON and RKN infection.

Taproot decline of soybean is caused by a novel *Xylaria* sp. that produces phytotoxins associated with foliar symptoms

T. G. GARCIA-AROCHA (1), P. Price (2), M. Tomaso-Peterson (3), T. Wilkerson (4), T. N. Spurlock (5), T. R. Faske (6), B. H. Bluhm (7), K. N. Conner (8), E. J. Sikora (9), R. Guyer (10), H. M. Kelly (10), T. Allen (11), V. Doyle (12), (1) Louisiana State University, Baton Rouge, LA, USA; (2) LSU AgCenter, Macon Ridge Research Station, Winnsboro, LA, USA; (3) Mississippi State University, Mississippi State, MS, USA; (4) MSU Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Stoneville, MS, USA; (5) University of Arkansas System Division of Agriculture Cooperative Extension, Monticello, AR, USA; (6) University of Arkansas System Division of Agriculture Cooperative Extension, Lonoke, AR, USA; (7) Department of Plant Pathology, University of Arkansas, Fayetteville, AR, USA; (8) Auburn University, Alabama Cooperative Extension System, Auburn, AL, USA; (9) Entomology and Plant Pathology, Auburn University, Auburn, AL, USA; (10) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA; (11) Delta Research and Extension Center, Mississippi State University, Stoneville, MS, USA; (12) Department of Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA, USA

Taproot decline (TRD) is a soybean disease caused by a *Xylaria* sp. that has become more frequently observed in the southern United States. Symptoms of TRD include interveinal chlorosis, followed by necrosis on leaves and on the tap and lateral roots. Elucidating the origin and etiology of this pathogen is relevant for the development of effective management practices. Phylogenetic analyses of 20 representative TRD-associated isolates from five southern states (AL, AR, LA, MS, and TN) suggest this taxon represents an undescribed species. Incorporating historical specimens from US herbaria, we found evidence that this pathogen has been present in the southern US for nearly a century and its emergence may represent a change in lifestyle from saprophyte to pathogen. Foliar symptoms of TRD resemble those of sudden death syndrome (SDS), which results from the translocation of phytotoxic compounds, produced by *Fusarium virguliforme*, from roots to leaves. Under controlled conditions, we tested the hypothesis that symptoms observed on soybean foliage are produced by systemic secondary metabolites released by the fungus in the roots. Stem cuttings challenged with cell-free culture filtrates showed lower chlorophyll content and reduced root growth, suggesting that the symptoms observed in leaves result from the translocation of phytotoxins from roots to the leaves. The recently developed protocol may be useful for screening soybean cultivars for resistance against TRD.

Evaluation of selected *Bacillus* spp. as biological control agents for Phytophthora Blight disease (*Phytophthora capsici*) in pepper

B. BHUSAL (1), M. T. Mmbaga (2), (1) Nashville, TN, USA; (2) Tennessee State University, Nashville, TN, USA

Phytophthora blight (*Phytophthora capsici*) is one of the most important diseases in horticultural crops including pepper (*Capsicum annuum*). Pepper growers rely heavily on pesticides and pesticide resistance is problematic. In addition, toxicity hazards to growers, consumers, non-target organisms and environmental contamination is of great concern. The objective of this study was to evaluate five isolates of *Bacillus* spp. (IMC8, PRT, PS, PSL, YNP5) as biological control agents for *P. capsici* disease management. While all five isolates promoted plant growth by increasing plant height, shoot weight, root length, root weight and chlorophyll content, three isolates IMC8, PS, PSL inhibited mycelial growth of *P. capsici* in *in-vitro* studies. The three isolates were evaluated further individually and in mixed populations in greenhouse environment using Phytophthora-infested soil. Mixtures of IMC8+PS, IMC8+PSL and IMC8+PS+PSL were similar to fungicide metalaxyl, in reducing *P. capsici* disease severity and in promoting plant growth. However, *Bacillus* isolate PSL displayed best potential in *P. capsici* disease management and plant growth promotion in Phytophthora-infested soil.

Cotton leafroll dwarf disease: An emerging viral disease on cotton in Georgia

S. BAG (1), A. Tabassum (1), N. Sedhain (1), K. Morgan (2), N. D. Suassuna (3), J. Whitaker (1), P. Chee (1), R. Kemerait (1), R. Nichols (4), P. Roberts (1), (1) University of Georgia, Tifton, GA, USA; (2) ABAC, Tifton, GA, USA; (3) EMBRAPA, Campina Grande, BRAZIL; (4) Cotton Incorporated, Cary, NC, USA

Cotton leafroll dwarf virus (CLRDV), which causes the cotton leafroll dwarf disease, is a single-stranded positive-sense, phloem restricted, virus efficiently transmitted by cotton aphids in persistent circulative manner. In the US cotton belt, the disease symptoms were first observed in AL in 2017 and confirmed in fall 2018. Subsequently, the virus has been confirmed in GA, FL, MS, and SC in 2018 and TX in 2019, indicating this virus is prevalent across the major cotton-producing states of the US. This viral disease could potentially impact the US cotton industry by significantly reducing the yield. The early symptoms associated with the disease include reddening of leaves, petiole, and leaves drooping. As the disease progress, more severe symptoms were observed including leaves rigidity, crinkling, low retention of squares, and fruits, resulting in yield loss. The symptoms are highly influenced by geographical locations, varieties, and cultural practices. Based on the whole genome sequence, the isolates from GA shared high levels of homology with the atypical strains of CLRDV reported earlier from Brazil and Argentina. In this collaborative effort, the UGA-Cotton team is working in a multidimensional, multidisciplinary approach to address the viral disease by understanding the epidemiology, etiology, disease spread, genomic composition/variability, developing tools for virus detection, symptomatology, vector transmission, and host-plant resistance.

Bacterial biocontrol agents against fungal and oomycete pathogens of rice and other field crops

L. Delgado (1), S. A. Winters (2), C. M. Rojas (3), A. ROJAS (3), (1) Universidad de los Andes, Bogota, COLOMBIA; (2) Entomology and Plant Pathology, University of Arkansas - Division of Ag, Fayetteville, AR, USA; (3) University of Arkansas, Fayetteville, AR, USA

Rice is the primary staple for more than half of the global population and is the second most important cereal worldwide. In the United States, Arkansas is the major producer of rice and this crop is affected by several fungal and bacterial diseases. The major diseases that affect the rice crop are the sheath blight whose causal agent is *Rhizoctonia solani*; rice blast by *Magnaporthe oryzae*; *Fusarium graminearum*, the causal agent of the fusarium head blight in wheat, but it also infects rice; and the oomycete *Pythium sylvaticum*, which causes seedling diseases in this cereal. Most management tactics for these diseases rely on the use of chemicals and tolerant cultivars. The use of novel strategies to manage these diseases could provide new approaches to minimize the impact on yields of this important crop. Recently, two potential biological control agents (BCA) were identified in Arkansas, *Pseudomonas fluorescens* and *Burkholderia cenocepacia*, showed inhibitory effect against the panicle blight pathogen *B. glumae*. The aim of this study was to determine the potential biocontrol activity of these BCAs against the prevalent fungal diseases on rice. These BCAs were challenged in dual culture assays against *R. solani*, *F. graminearum* and *P. sylvaticum* on three different media optimizing the biocontrol effect. The results showed an antagonistic activity of *B. cenocepacia* against the panel of fungi evaluated, suggesting that this bacteria could be used as a biocontrol agent.

Evaluation of host plant defense inducers for the management of plant pathogens in two North Carolina cropping systems

A. LLANOS MELO (1), R. Kreis (2), S. M. Villani (2), (1) North Carolina State University, Mill River, NC, USA; (2) North Carolina State University, Mills River, NC, USA

Inducers of systemic acquired resistance (SAR) generally trigger the production of salicylic acid and the activation of plant defense genes, inhibiting or slowing the development of pathogens. These compounds are promising tools for plant disease management, but a full understanding of their performance and mechanisms of action is necessary. A series of studies evaluated the performance of the SAR inducers Actigard (acibenzolar-S-methyl) and LifeGard (*Bacillus mycoides* isolate J) against boxwood blight (*Calonectria pseudonaviculata*) in boxwood cv. 'Justin Brouwers' and fire blight (*Erwinia amylovora*) in apple 'Rome Beauty.' An additional product, Regalia (*Reynoutria sachalinensis* extract) was tested on apple. Products were evaluated under different application strategies and disease incidence was evaluated on boxwood shoots and on apple blossoms. Plants treated with plant defense inducers had less

disease than the control in both cropping systems. Boxwood treated with drench applications of Actigard had significantly less disease than untreated programs ($\alpha = 0.05$), while applications of Actigard, LifeGard, and Regalia significantly reduced blossom blight incidence in apple ($\alpha = 0.05$). The expression of pathogenesis related-genes (PR genes; PR-1, PR-2, and PR-8) was evaluated for apple. The level of expression of PR-2 and PR-8 genes in plants treated with Actigard and Regalia increased significantly related to the control one day after application ($\alpha = 0.05$).

Susceptibility of hemp varieties to leaf spot in Tennessee

Z. R. HANSEN (1), H. M. Kelly (2), M. Cartwright (3), E. Walker (4), (1) University of Tennessee, Knoxville, TN, USA; (2) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA; (3) University of Tennessee, Dyersburg, TN, USA; (4) Burley Stabilization Corporation, Springfield, TN, USA

An estimated 25,000 acres of hemp were planted in Tennessee in 2019, nearly all of which was produced for cannabidiol (CBD). Hemp leaf spot, caused by several fungal pathogens including *Curvularia/Bipolaris* sp., *Cercospora* sp., and *Alternaria* sp., was widespread and, in some fields, severe. The objective of this study was to evaluate hemp-for-CBD varieties for susceptibility to leaf spot. Field trials were conducted at three locations in 2019: the University of Tennessee (UT) Greenville Research and Education Center (REC) in Greenville, TN (GREC), the UT Highland Rim REC in Springfield, TN (HRRREC), and an on-farm location in Jackson, TN (Jackson). The experimental design at each location was a randomized complete block design with four replications at GREC and HRRREC and six replications at Jackson. Plots consisted of two rows, five plants per row. Trials were planted between 17–28 Jun and rated for disease between 2–7 Oct. Leaf spot incidence and severity were recorded. Differences among varieties were observed at each location, and differences were generally in agreement across locations. The varieties with the lowest disease index values were: 'Late Sue', 'T-Rex', 'Franklin', 'CBD Therapy', 'Super CBD', 'OG', 'Frosted Lime', and 'ACDC'. The following varieties had the highest disease index values: 'Tangerine', 'Baoux', 'T1', and 'Cherry Tart'. In general, disease index values tended to be lower for later-maturing varieties than early-maturing varieties.

Evaluating candidate anthracnose disease resistance genes in sorghum

L. STUTTS (1), W. Vermerris (1), H. Cuevas (2), (1) University of Florida, Gainesville, FL, USA; (2) USDA, Mayaguez, PR, USA

The cereal crop *Sorghum bicolor* has a high tolerance to abiotic stresses, making it attractive for cultivation in environments generally unsuitable for food crops. A constraint to sorghum cultivation in Florida is the prevalence of the anthracnose-causing fungal pathogen *Colletotrichum sublineola*. A previous mapping study identified two quantitative trait loci which contain over 50 candidate anthracnose resistance genes. Virus-induced gene silencing (VIGS) and gene expression analyses via RNA sequencing and quantitative RT-PCR are being used to evaluate the contribution of these genes to anthracnose resistance. VIGS is being used to downregulate 12 candidate genes in resistant sorghum, and excised leaf assays are subsequently used to assay disease resistance. Gene expression is assayed with quantitative real-time PCR (qRT-PCR). RNA sequencing analyses in inoculated and mock-inoculated plants of resistant and susceptible genotypes points to a cluster of germin-like genes, along with several genes with leucine-rich repeat domains, as conferring disease resistance. Staining with the compound 3,3'-diaminobenzidine (DAB) has been used to evaluate the potential role of germin-like proteins in the production of reactive oxygen species (ROS). Results obtained so far indicate that anthracnose resistance in sorghum cultivar Bk7 appears to derive from several genes on chromosome 7, and one gene on chromosome 9, which may respectively be involved ROS production and signaling.

The occurrence of *Meloidogyne floridensis* on collard greens in Georgia, USA

J. Marquez (1), F. Forghani (2), A. HAJIHASSANI (3), (1) Graduate Student, GA, USA; (2) Postdoctoral Associate, GA, USA; (3) University of Georgia, Tifton, GA, USA

Root-knot nematodes (RKN, *Meloidogyne* spp.) are widely distributed in vegetable fields of southern Georgia. During a survey of plant-parasitic nematodes in vegetable crops in 2018, a composite soil sample was taken from a collard greens (*Brassica oleracea* var. *acephala*) field in Dooly County, Georgia. Nematodes were extracted from the soil and RKN detected to genus level by typical morphological features of second-stage juveniles. Subsequently, the soil sample was potted for RKN culturing on tomato (cv. Rutgers). After 10–12 weeks, multiple adult female nematodes were isolated from galled roots for genomic DNA extraction by smashing a female in 25 μ L water. DNA extractions were subjected to PCR using three primer sets (TRNAH/MRH106, C2F3/1108, and NAD5F/NAD5R) which amplify regions in the cytochrome c oxidase subunit II (COII), large subunit 16S rDNA (16S) and NADH dehydrogenase subunit 5 (NADH5) gene. The PCR products were purified and subjected to Sanger sequencing followed by DNA homology analysis with BLAST. DNA sequence data confirmed the presence of *M. floridensis* with highest hit number of 100 (COII), 98.2 (COII and 16S) and 97.5% (NADH5) identity with the *M. floridensis* reference genomes. A pathogenicity test is currently underway to determine if collard greens is a host for the nematode. This is the first report of *M. floridensis* (peach root-knot nematode) in Georgia, suggesting that the nematode is an emerging pathogen in southeast US.

Decomposition treatments for reducing *Nothopassalora personata* inoculum in peanut fields

H. R. BECTON (1), E. Cieniewicz (2), K. Kirk (1), D. J. Anco (1), (1) Clemson University, Blackville, SC, USA; (2) Clemson University, Clemson, SC, USA

Nothopassalora personata is the causal agent of late leaf spot (LLS) of peanut. The objective of this study was to examine peanut residue decomposition treatments applied in the fall or spring to reduce subsequent LLS infections. Decomposition treatments included urea ammonium nitrate (UAN) and UAN plus a microbial stubble digester (SD25) (fall) or UAN and UAN plus Bond Max surfactant (BM) (spring). Non-treated checks (NTC) were included in both experiments, and fungicides were not applied during the growing season. Soil samples were taken before treatment application and within 30 days after planting (DAP). Observations were made bi-weekly for LLS incidence, severity, and defoliation. Examined soil fertility measurements were not affected by decomposition treatments. Fall application of UAN plus SD25 exhibited significantly less (5.8%) LLS incidence 93 DAP compared to NTC and UAN ($P = 0.003$), though further assessments were not significantly different. Incidence, severity, and defoliation from LLS were significantly different among spring-applied treatments at 105 through 141 DAP. Peanut treated with UAN plus BM generally had significantly less LLS severity and defoliation when compared to UAN, with defoliation at 141 DAP corresponding to 64.8 and 90.5%, respectively. While additional data are required, decomposition treatments represent a new and economical tool for integrated management for LLS on peanut that is compatible with currently used equipment.

Transmission of *Rose rosette emaravirus* (RRV) through non-vector related methodologies

M. K. SHIRES (1), J. Corser (1), J. Ueckert (2), K. Ong (2), (1) Texas A&M University, College Station, TX, USA; (2) Texas A&M AgriLife Extension Service, College Station, TX, USA

RRV, a negative sense RNA virus with seven segments, has been causing issues on its host, roses, since the 1940's. Transmission of the virus was demonstrated in the 1950's by bud grafting; possible root transmission was suggested. As the virus has continued to spread across the country, the question of alternate means of transmission has been posed. RRV does have an eriophyid mite vector; However high amounts of RRV being found in previously disease free areas suggests that the virus may be introduced through other types of transmission such as root grafting, bud grafting, seed transmission and mechanical transmission. Transmission research was completed on roots by planting virus positive plants in the same pot as virus free plants and observing for symptoms of RRV infection. Grafting was completed through a T-bud method. Seed transmission involved the collection of seeds from RRV symptomatic plants. Mechanical transmission was completed by utilizing mechanical inoculation techniques for other viruses. After PCR testing, limited amounts of RRV transmission were observed in both root and seed studies. This suggests that RRV could be spread in production fields by roots contacting each other due to plant spacing as well as RRV being distributed by seed spread. Bud grafting and mechanical transmission have not yet yielded any positive PCR results, but this is thought to be due to suppression of RRV during summer heat; testing will continue at three month intervals.

Evaluating the response of new soybean germplasm to environmental conditions that promote reduced grain quality

J. FOMBA (1), M. Tomaso-Peterson (2), D. Cook (3), T. W. Allen Jr. (4), J. R. Smith (5), T. Wilkerson (6), (1) Mississippi State University, Stoneville, MS, USA; (2) Mississippi State University, Mississippi State, MS, USA; (3) Mississippi State University, Verona, MS, USA; (4) Mississippi State Univ, Stoneville, MS, USA; (5) USDA-ARS, Stoneville, MS, USA; (6) MSU Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Stoneville, MS, USA

Phomopsis longicolla (PL) is a fungus responsible for several soybean diseases. Most notably, PL causes Phomopsis seed decay (PSD), a major cause of reduced grain quality in the southern U.S. some years. Symptoms associated with PSD include shriveled, cracked, elongated, and chalky white soybean kernels. Warm temperatures and high humidity during pod fill and beyond maturity are considered necessary for PSD development. The objective of this research was to evaluate 21 soybean entries in PL-inoculated situations under environmental conditions conducive to PSD development. In 2019, soybean plots were established under two shelters consisting of plastic-covered greenhouse frames. Each shelter contained the 21 entries as single-row plots, 3.6 m long, replicated three times. Soybean plants in all shelters were inoculated with a PL conidial suspension at R5. Shelter 2 received a fungicide application consisting of 293 ml/ha of trifloxystrobin + prothioconazole at R7 while shelter 1 remained non-treated. Intermittent, overhead irrigation was conducted in both shelters beginning at R5 (≈ 100 h). Plots were harvested using a one-row combine; however, harvest was delayed beyond maturity. Plot weight (g) and grain damage (%) evaluations were made post-harvest. Symptoms associated with PSD were observed regardless of entry. No significant differences were observed in plot yield among entries in either the fungicide-treated ($p = 0.1163$) or non-treated ($p = 0.2092$) shelter.

Comparative transcriptome analysis of two contrasting maize inbred lines provides insights on molecular mechanisms for stalk rot resistance

A. SALCEDO (1), J. Al-Haddad (2), C. R. Buell (2), F. Trail (2), E. Góngora-Castillo (3), L. M. Quesada (4), (1) North Carolina State University, Raleigh, NC, USA; (2) Michigan State University, East Lansing, MI, USA; (3) Centro de Investigación Científica de Yucatán, Chuburná de Hidalgo, MEXICO; (4) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Maize stalk rot caused by *Fusarium graminearum* is one of the most destructive plant diseases causing severe crop loss and dangerous mycotoxin contamination for humans and livestock. The development of resistant varieties is considered a desirable strategy to control the disease. However, the tissue-specific defense mechanism in maize against *F. graminearum* is largely unknown and strongly influenced by environmental conditions. In this study, we have analyzed and compared the phenotype and transcriptome of a resistant

and a susceptible maize inbred line in response to *F. graminearum* during a time-course infection using RNA-seq technology. We evaluated the influence of two environmental conditions on the resistant phenotype and identified 1,366 differentially expressed genes (DEGs) at 2, 14- and 28 days post-infection. Analysis of expression abundance and gene ontology enrichment among resistant and susceptible transcriptomes suggest an important role of gene regulation with nearly 50% of DEGs associated to catalytic activity function. We validated the transcriptome analysis evaluating the expression patterns of representative DEGs by RT-qPCR confirming the RNA-seq results and the environmental influence on resistance. Our findings provide important information regarding the genetic basis of maize resistance to *F. graminearum* and a first step towards development of resistant varieties.

Black rot resistance in sweetpotato (*Ipomoea batatas*): A case of age-related resistance

C. H. PARADA ROJAS (1), K. Pecota (2), C. Yench (3), L. Quesada-Ocampo (4), (1) NC State University, Raleigh, NC, USA; (2) North Carolina State Univ, Raleigh, NC, USA; (3) North Carolina State University, RALEIGH, NC, USA; (4) North Carolina State University, Raleigh, NC, USA

In 2015, the soilborne pathogen *Ceratocystis fimbriata* reemerged threatening sweetpotato production in the United States. Since chemical control is limited for export markets, deployment of host resistance represents a desirable tool to decrease losses caused by *C. fimbriata*. Here, we report a resistance screening at the cambium ring, parenchyma, and slip level in a diverse panel of 48 sweetpotato cultivars, advanced breeding lines, and wild relative accessions. In addition, we assessed 'Covington' roots with different total cross-section root areas for susceptibility to an isolate of *C. fimbriata*. None of the cultivars examined at the storage root level were completely resistant, with most cultivars exhibiting similar levels of susceptibility. For the storage root assay, Jewel and Covington were categorized as the least susceptible and significantly differing from White Bonita, the most susceptible cultivar. For the slip assay, Burgundy and *I. littoralis* displayed lower below ground incidence as compared to Hopi, the most susceptible cultivar. Black rot cross-sectional lesion ratio was negatively correlated ($\rho = -0.82$) with total cross-section root area, suggesting a strong case of age-related resistance. Our research highlights a potential resistance mechanism against *C. fimbriata* and assists management and breeding programs in limiting losses in sweetpotato.

Physiological and molecular-genetic characterization of basal resistance in sorghum

R. SAMIRA (1), J. Kimball (2), L. F. Samayoa Lopez (3), G. Stacey (4), P. Balint-kurti (5), (1) Texas Tech University, Lubbock, TX, USA; (2) University of Minnesota, St. Paul, MN, USA; (3) North Carolina State University, NC, USA; (4) University of Missouri, Columbia, MO, USA; (5) North Carolina State University, Raleigh, NC, USA

Sorghum (*Sorghum bicolor*) is widely used for human and animal consumption as well as biofuel production. It is drought and heat tolerant and requires less nutrients for growth than many other crops. In this project, we emphasized on understanding the genetics associated with the basal immunity of Sorghum. Basal immunity is considered the first line of defense in the plant. Microbe-associated patterns are recognized by pattern recognition receptors present on the plant cell surface, triggering a signal transduction that activates a suite of defense genes. An association mapping population consisting of 492 natural Sorghum lines was assayed for production of reactive oxygen species (ROS) in response to flg22 (bacterial MAMP) and chitin (fungal MAMP). Based on their response and genetic variations, putative QTL associated with basal immunity was determined. These lines were also scored for disease (Target Leaf Spot, TLS) susceptibility in the field to further determine whether the disease resistance and MAMP response are under shared genetic control. We have identified one strong association of TLS at chromosome 5 and moderately significant correlation between strong flg22 response to low TLS resistance.

Physiological effects of soybean cyst nematode on real time N₂-fixation activity, and leaf growth parameters of three soybean lines

R. AKINRINLOLA (1), H. M. Kelly (2), T. Sinclair (3), A. Shekoofa (4), (1) University of Tennessee Knoxville, Jackson, TN, USA; (2) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA; (3) Crop Science Department, North Carolina State University, Raleigh, NC, USA; (4) University of Tennessee, Jackson, TN, USA

Soybean cyst nematode (SCN) causes over \$1 billion soybean yield loss annually in the United States. The nematode can lower soybean N₂-fixation activity (NFA), but the dynamics of the NFA reduction and effects on soybean growth parameters are unknown. A greenhouse study was performed to track the effects of SCN on NFA and leaf growth over time on three soybean lines. The soybean lines (PI88788, Williams 82, and Ellis) were treated with 10000 SCN eggs/seedling at planting as treatment, and untreated seedlings were grown as control. Eight replicates were planted per soybean line. Starting from 20 days after planting, data on NFA of soybean (rhizosphere ethylene concentration) were taken twice per week. Leaf chlorophyll concentrations, area, and nitrogen content were also determined. The NFA of Williams 82 was significantly reduced by SCN on average across the sampling period, while that of PI88788 was significantly reduced by SCN on two sampling dates, but not significantly across the sampling period. Percentage decrease in NFA on PI88788 and Williams 82 ranged from 7 to 26% and 12 to 25%, respectively, compared to untreated. Leaf chlorophyll concentrations and leaf area of Williams 82 were significantly reduced by SCN, but that of PI88788 and Ellis was not affected. Leaf nitrogen content was not affected by SCN on any of the soybeans. This study provides insights into the physiological effects of cyst nematodes on plant health and growth.

Identification of effective fungicides for control of black rot, caused by *Ceratocystis fimbriata*, in sweetpotato for domestic and export markets

H. COLLINS (1), L. M. Quesada (2), (1) North Carolina State University, Raleigh, NC, USA; (2) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

North Carolina is the largest producer of sweetpotatoes in the United States, with over 75,000 acres harvested every year and approximately 30–40% of the crop exported. *Ceratocystis fimbriata*, the causal agent of black rot, is an economically important postharvest pathogen of sweetpotato. Few active ingredients are available and labeled for disease control in sweetpotato, and even fewer products are allowed for export due to changes in maximum residue levels. To identify fungicides to manage black rot that can be used for domestic or export markets, registered and unregistered products were evaluated for efficacy. Sweetpotato roots were artificially inoculated with *C. fimbriata* and placed onto a packing line for treatment applications. Fungicide sprays were applied using a compressed air pressurized sprayer delivering 0.69 gal/2,000 lb of roots. Roots were then incubated at 24°C for 21 days and rated for disease incidence at 7, 14, and 21 days. Our results revealed that Mertect 340F and Quash provided significant reductions in black rot incidence at 21 days. Treatments including Mertect, Quash, and Stadium also provided significant reductions in black rot incidence at 14 days. Findings of this study are an important step towards increasing the number of registered products for postharvest disease control in sweetpotato to support the continued expansion of export markets.

Pseudomonas chlororaphis type VI secretion systems: Roles in rhizosphere dynamics and plant microbe interactions

E. BOAK (1), L. S. Pierson III (2), E. A. Pierson (3), (1) College Station, TX, USA; (2) Texas A&M University, Plant Pathology and Microbiology, College Station, TX, USA; (3) Texas A&M University, College Station, TX, USA

Many plant-beneficial bacteria have one or more Type VI Secretion Systems (T6SS), but their functions in plant-associated niches, including interactions with the host plant, are poorly understood. T6SS are bacterial machines studied predominantly in human and plant pathogens as a mechanism for bacterial competition and killing, and for the transmission of effector proteins critical for virulence into eukaryotic cells. Within some bacteria, distinct T6SS have different roles allowing for multiple functionalities. The present work focuses on *Pseudomonas chlororaphis* 30-84, a well-studied wheat rhizosphere-colonizing plant-beneficial bacteria strain with two distinct T6SS that appear to perform non-redundant functions. The role of each T6SS (T6SS-1, T6SS-2) in microbial competition and persistence in the rhizosphere, and host plant recognition and immune response was examined. Mutants deficient in the T6SS-2, but not the T6SS-1, were less competitive than the wild type against other bacteria in competition assays *in vitro* and on wheat roots. Mutants deficient in the T6SS-2 were less effective rhizosphere colonists than the wild type in repeat-harvest assays. Preliminary data using *Arabidopsis* microbial recognition reporters indicated functional T6SS are important for plant recognition of bacteria and pattern-triggered immunity response. These data indicate roles for the T6SS of plant-beneficial bacteria in rhizosphere dynamics and plant microbe interactions.

Formation, viability and survival of *Pseudoperonospora cubensis* oospores under field conditions in southeastern United States

I. KIKWAY (1), A. P. Keinath (2), P. S. Ojiambo (3), (1) North Carolina State Univ, Raleigh, NC, USA; (2) Coastal Research and Education Center, Clemson University, Charleston, SC, USA; (3) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is an important disease of cucurbits globally. In regions with harsh winters, overwintering oospores could serve as an initial inoculum source for early disease onset. While oospore formation has been documented in the United States under controlled conditions, their occurrence and survival under field conditions has not yet been established. In fall 2018, 470 diseased cucumber and cantaloupe leaves were collected from fields in Rocky Mount, NC and Charleston, SC. Collected leaves were clarified in an ethanol and acetic acid solution and observed under a compound microscope for the presence of oospores. To assess survival under field conditions, oospores were first produced in the lab by crossing compatible A1 and A2 isolates. Oospore infested leaves were then placed in the field in November 2018 and sampled monthly from January to April to assess oospore survival during the winter. About 4.5% of diseased leaves sampled from fields in NC and SC had oospores with an average of 418 oospores per cm² of leaf tissue. Baseline viability of oospores prior to field exposure was 70%. Subsequent monthly assays from February through May 2019 showed oospore viability levels at 45%, 23%, 30% and 22%, respectively. Oospore formation under field conditions and their viability and survival during the winter suggest that oospores could potentially play a role in CDM epidemiology in the southeastern United States.

Response of snap bean genotypes to two distinct begomoviruses associated with leaf crumple disease

S. KAVALAPPARA, B. Dutta, R. Srinivasan, University of Georgia, Tifton, GA, USA

Leaf crumple is an emerging disease complex that severely limits the production of snap beans in Southern USA. Cucurbit leaf crumple virus (CuLCrV) and Sida golden mosaic Florida virus (SiGMFV) are two whitefly (*Bemisia tabaci*) transmitted begomoviruses that have recently been associated with snap beans in Georgia. In field conditions, the disease is identified based on symptoms of yellowing, crumpling and severe stunting. Under controlled greenhouse conditions, it was observed that the symptoms produced by these two

viruses are quite different on snap beans. SiGMFV produced yellow mosaic symptoms that were clearly visible as early as ten days post-inoculation (DPI) on susceptible cultivars. CuLCrV caused the gradual shortening of internodes and leaves leading to a bunchy top appearance on the same susceptible lines, which was visible after 20 DPI. When both viruses were inoculated together, the symptoms of SiGMFV were more prominent than that of CuLCrV. The cultivars, Prevail and Sybaris showed higher levels of resistance to SiGMFV and supported lower virus titer compared to the susceptible lines when evaluated via whitefly-mediated transmission. However, they showed a susceptible reaction to CuLCrV suggesting the mechanisms of resistance to these two viruses are distinct. The difference in characteristics of the two viruses on snap beans is an important factor to consider while developing and refining management practices based on host resistance.

Identification and management of *Phytophthora vexans* on *Ginkgo biloba*

M. PANTH (1), T. Simmons (2), F. Avin (2), F. Baysal-Gurel (2), (1) Tennessee State University, Mc Minnville, TN, USA; (2) Tennessee State University, McMinnville, TN, USA

Ginkgo (*Ginkgo biloba* L.) plants grown in container and field in Tennessee have shown root and crown rot symptoms with reddish/dark brown lesions in 2017 and 2018. The objective of this study was to identify the pathogen affecting ginkgo in nursery production and develop management recommendations. Isolations were done from the infected roots. Several *Phytophthora*-like colonies with spherical zoospores, ovoid to globose oogoni, and whitish mycelium, were isolated on PARPH-V8. To confirm the identity of the pathogen, total DNA was extracted using the PowerSoil DNA Isolation Kit directly from 3-day-old cultures. PCR and sequencing were done to confirm the pathogen identification followed by pathogenicity test to complete Koch's postulates. Following the identification of *Phytophthora vexans*, the efficacy of fungicides, biofungicides, host plant defense inducers, and fertilizer against *P. vexans* was assessed in greenhouse trials. Pots were inoculated with *P. vexans* grown on PARPH-V8. Ginkgo roots were assessed for *Phytophthora* root rot disease severity using a scale of 0–100% roots affected. At the end of two greenhouse trials, Segovis, Pageant, Subdue MAXX, Empress, Stargus and Orchestra were consistently more effective in reducing *Phytophthora* disease severity than other treatments and the non-treated, inoculated control. This comparative study will help on developing management recommendations for newly reported *Phytophthora* root and crown rot disease of ginkgo.

Characterization, detection and management of emerging tomato brown rugose fruit virus infecting greenhouse tomatoes in the U.S.

B. CHANDA (1), M. Galvez (2), S. Nunziata (3), Y. Rosado-Rivera (4), A. Gilliard (5), K. S. Ling (5), (1) USDA ARS, Charleston, SC, USA; (2) Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, USA; (3) USDA-APHIS-PPQ-S&T-CPHST, Beltsville, MD, USA; (4) USDA-APHIS-PPQ S&T CPHST, Beltsville, MD, USA; (5) USDA-ARS, Charleston, SC, USA

Tomato brown rugose fruit virus (ToBRFV), first identified in 2014 in the Middle East, has caused serious outbreaks in greenhouse tomatoes of many countries in Asia, Europe and North America. With its seed-borne, easy mechanical transmission and resistance breaking to the popular *Tm2²* gene, ToBRFV could potentially cause serious economic losses to tomato industries worldwide if not managed timely. In the U.S., the first ToBRFV outbreak on tomato was reported in 2018 in a greenhouse facility in California. In the current study, using high throughput sequencing, a complete genome sequence of the US isolate of ToBRFV was obtained. The high nucleotide sequence identity (99%) to other known ToBRFV isolates around the world suggests a high possibility in sharing the same or similar origin. In a comparative evaluation of potential host ranges among tomato-infecting tobamoviruses, despite of many similarities, some unique host species were also identified for each respective virus. Due to cross serological reactivity among tomato-infecting tobamoviruses, a species-specific real-time RT-PCR was developed for ToBRFV. Disinfectants that are capable of deactivating virus infectivity to prevent mechanical transmission were evaluated and several promising disinfectants were identified. Finally, nearly 500 accessions of tomato germplasm and cultivars were screened for their potential in resistance against ToBRFV.

Assessment of nematicides for corn grown in fields infested with *Belonolaimus longicaudatus* and *Meloidogyne incognita*

R. KEMERAIT (1), M. Toews (2), (1) University of Georgia, Tifton, GA, USA; (2) UGA Department of Entomology, Tifton, GA, USA

Corn production in Georgia is limited by nematodes, to include southern root-knot (*Meloidogyne incognita*) and sting (*Belonolaimus longicaudatus*). The objective here was to compare efficacy of a nematicide seed treatment (NemaStrike), a granular product (Counter 20G) and liquid products (Propulse and Luna Privilege). Trials were established in Tifton, GA in April 2019. One was planted to an area affected by *B. longicaudatus* and the other to an area affected by *M. incognita*. Treatments were applied to Dekalb 67-44 in a randomized complete block design with 4 replications. Treatments included base-treated seed, NemaStrike, Propulse (4.0, 6.0, and 8.0 fl oz/A), Luna Privilege (3.0 fl oz/A), Nematstrike + Propulse (6 fl oz/A) or Luna Privilege (3 fl oz/A), and Counter 20 G (5 lb/A). Plots were rated for height and yield. Where sting nematodes occurred, plants treated with Counter 20G, Propulse + NemaStrike, and Luna Privilege were taller than plants treated with base treatment or NemaStrike. Where *M. incognita* occurred, yields from plots treated with Propulse or Counter 20G were 15-19 bu/A and 13-16 bu/A greater than from plots with base-seed treatment or NemaStrike, respectively. Where *B. longicaudatus* was occurred, all except NemaStrike significantly out-yielded the base-seed treatment; the largest yield advantages were in plots treated with Propulse (8 fl oz/A) and Counter 20G, which out-yielded the base-seed treatment by 47 and 55 bu/A, respectively.

Identification of molecular biomarkers associated with reniform nematode (*Rotylenchulus reniformis*) resistance in soybean

J. E. FULTZ WILKES (1), P. A. Agudelo (2), (1) Clemson University, Central, SC, USA; (2) Clemson University, Plant and Environmental Sciences Department, Clemson, SC, USA

Reniform nematode (*Rotylenchulus reniformis*) is a yield-limiting pathogen of soybean in the Southeastern region of the United States. Studies have identified soybean germplasm with resistance to reniform nematode and only a few have explored the soybean genome for quantitative trait loci (QTL) linked to reniform nematode resistance. The objective was to identify high resolution single-nucleotide polymorphism (SNP) biomarkers that correlate with soybean host suitability to reniform nematode using genotyping-by-sequencing (GBS). A population of 250 recombinant inbred lines developed from a cross between a reniform nematode resistant and susceptible cultivar was utilized to map host suitability. Resistance was quantified using an optimal univariate cluster analysis on both normalized and transformed raw reproduction indices. Two QTLs were found in association with reduced reproduction of reniform nematode, one on chromosome 11 and one on 18, in regions previously reported. A third QTL on chromosome 18 was significantly associated with the phenotype, however inversely to the other two QTLs, correlating with an increase in nematode reproduction or host suitability. Two QTL regions were found using $\log_{10}(x)$ transformed phenotype data while the third QTL was found using non-transformed data. These genetic markers can be used by soybean breeders in marker-assisted selection to enhance efforts in selecting and employing lines with known resistance to reniform nematode.

Development of a novel isothermal molecular assay with an internal control to detect Tomato brown rugose fruit virus

R. LI (1), B. Davenport (1), S. Zhang (1), K. Schuetz (1), K. S. Ling (2), (1) Agdia, Inc., Elkhart, IN, USA; (2) USDA-ARS, Charleston, SC, USA

Tomato brown rugose fruit virus (ToBRFV) is a new Tobamovirus first reported in Jordan in 2015. The subsequent reports from Asian, American, and European countries indicate that ToBRFV has already been spread throughout the world. The serious damage to tomato and pepper plants, the resistance-breaking ability, and the seed-borne nature of ToBRFV have caused a huge concern within government organizations, seed production industries, and tomato growers worldwide. In order to facilitate reliable detection of ToBRFV in tomato and pepper plants and thus effectively manage ToBRFV disease, Agdia is developing ToBRFV-specific ELISA and ImmunoStrip assays as well as a ToBRFV-specific isothermal AmplifyRP assay. The goal for the AmplifyRP assay is to target two virus-specific genes and one host gene as an internal control to reliably detect ToBRFV from crude extracts of leaves, fruits, and seeds. The primers and probes were designed from four conserved viral genomic regions. The screening experiments showed that two primer/probe combinations located in two separate genomic regions exhibit specific detection for ToBRFV, but no cross-reactions to ten other Tobamoviruses such as Tobacco mosaic virus, Tomato mosaic virus, Tomato mottle mosaic virus, and Pepper mild mottle virus. The optimization of primer and probe combinations to achieve a sensitive and reliable detection for ToBRFV will be reported.

Surveying quinone outside inhibitor (QoI) resistance in peanut late leaf spot (*Nothopassalora personata*) in eastern North Carolina in 2018

T. SCHAPPE (1), B. B. Shew (2), L. D. Thiessen (1), (1) North Carolina State University, Raleigh, NC, USA; (2) NC State University, Raleigh, NC, USA

Late leaf spot caused by the endemic fungal pathogen *Nothopassalora personata* is a severe disease of peanut (*Arachis hypogaea* L.) in the southeastern and Virginia-Carolinas regions of the United States. QoIs are a class of fungicides that block the electron transfer site of the cytochrome bc 1 complex, inhibiting cellular respiration. Three non-synonymous mutations in the cytochrome b gene are associated with resistance to QoIs, ranging from moderate to complete: F129L, G137R, and G143A. QoIs provided excellent late leaf spot control for over a decade, but recent apparent control failures suggest that QoI resistance may be present in *N. personata* populations in North Carolina. Our objective was to determine the relative frequency and geographic distribution of QoI resistance in *N. personata* in North Carolina. We collected leaves from 48 infected peanut plants from 33 locations in eastern NC in 2018 and extracted DNA from 8 lesions per plant. We performed PCR to amplify the regions around the QoI mutations, sequenced the resulting amplicons using Sanger sequencing, and aligned the sequences to identify polymorphisms among samples. For G143A, 30 (62.5%) samples were mutant, 12 (25%) were wild-type, and 6 (12.5%) were mixed wild-type and mutant; all samples were wild-type for F129L and G137R. Our results show a high frequency of QoI resistance among isolates collected across NC, and selection of other fungicide classes is likely necessary to avoid control failures.

Optimal timing for preventative pythium root rot management

H. HAMPY, North Carolina State Univ, Raleigh, NC, USA

Pythium root rot is a detrimental disease to creeping bentgrass golf course putting greens in the Southeastern United States. The disease is typically managed by spraying fungicides throughout the summer months. Yet, the timing for fungicide applications for preventative management is poorly understood. Therefore, the objective of this study is to determine the efficacy of different fungicide application timings on turfgrass quality in order to best manage Pythium root rot symptoms on creeping bentgrass putting greens. Cyazofamid

(Segway SC) was initially applied on March 14, April 10, May 8, June 5, July 3, and August 1. The first treatment received 6 applications starting in March, the second treatment received 5 applications starting in April, the third treatment received 4 applications starting in May, the fourth treatment received 3 applications starting in June, and the fifth treatment received 2 applications starting in July. One quarter-inch of post-application irrigation was applied immediately following the fungicide application. Applications starting in March, April and May, or before temperatures reach 65°C resulted in improved turfgrass quality when compared to the applications sprayed in June and July, or after soil temperatures increased above 65°C.

***Pythium* spp. infecting winter field and cover crops in North Carolina**

E. REEVES (1), J. P. Kerns (2), C. Cowger (3), B. B. Shew (4), (1) NC State Univ, Raleigh, NC, USA; (2) North Carolina State University, Raleigh, NC, USA; (3) USDA-ARS Plant Science Unit, Raleigh, NC, USA; (4) Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, USA

Annual winter field and cover crops grown in North Carolina include wheat, clary sage, rye, rapeseed and winter pea. Poor stands, mild to severe stunting, and root rot are common problems in these crops during wet years. Symptoms in winter wheat recently were associated with infection by several *Pythium* spp. (Lookabaugh et. al, 2017). To further characterize the prevalence and causes of *Pythium* root rot in wheat and other winter crops, plants displaying symptoms of stunting and root rot were collected in the winter of 2018–2019. Among the 100 isolates of *Pythium* collected from wheat in eight counties in NC, 34 were *P. irregulare*, 18 were *P. vanterpoolii*, and 17 were *P. spinosum*. Seedling pathogenicity assays were performed with a subset of isolates. Symptoms ranged from slight to severe root necrosis. Inoculation with *P. irregulare*, *P. spinosum*, or both species caused severe root rot under all conditions tested. *Pythium* spp. also were identified from clary sage, rye, rapeseed, and winter pea. Aggressiveness of eight species from clary sage was compared at 18°C and 28°C. *P. irregulare*, *P. sylvaticum*, and *P. spinosum* were the most aggressive species at 18°C, but not at 28°C. Results suggest that temperature influences the aggressiveness of *Pythium* spp. infecting winter field and cover crops.

Evaluation of Salibro as a new nematicide against major plant-parasitic nematodes

B. LAWAJU, K. S. Lawrence, Entomology and Plant Pathology, Auburn University, Auburn, AL, USA

Salibro (Fluazaindolizine) was evaluated *in vitro* and *in planta* for its efficacy on *Meloidogyne incognita*, *Rotylenchulus reniformis*, and *Heterodera glycines* egg hatch, juvenile (J2) mortality, and subsequent reduction of nematode population density in plants. In all tests, Salibro was applied at 1, 5, 50, and 250 ppm and tests were arranged as RCBD with 4 or 8 replications. *In vitro* tests were conducted in 12-well tissue culture plates. Nematode egg hatch and J2 mortality data were collected for 7 days. Greenhouse tests were conducted with *M. incognita* and *R. reniformis* on DP 1558 NR B2RF and *H. glycines* on Williams 82 cultivars. Salibro and 3,000 nematode eggs in 150 cm³ of soil were added at planting. Plant and nematode data were collected at 35 DAP. All data were analyzed with SAS 9.4 using PROC GLIMMIX and LS means were compared using Tukey-Kramer's method. The egg hatch assay indicated Salibro at 250 ppm inhibited egg hatch over 7 days of exposure, but J2s were extensively affected within 24 hours of exposure in all rates. All the rates of Salibro had similar plant height and biomass. The nematode population density of *M. incognita* and *R. reniformis* were significantly reduced by 5, 50, and 250 ppm of Salibro, whereas *H. glycines* required 250 ppm to have similar effect. The results demonstrated that *M. incognita* and *R. reniformis* populations were significantly reduced with lower concentration of Salibro compared to *H. glycines* under greenhouse conditions.

Formulated magnesium nanomaterials as novel alternatives to bacterial spot of tomato disease management

Y. Y. LIAO (1), A. L. Strayer-Scherer (2), Z. Huang (3), S. Santra (3), J. C. White (4), R. De La Torre-Roche (4), Q. Fan (5), S. Da Silva (5), G. E. Vallad (6), J. H. Freeman (5), J. B. Jones (1), M. L. Paret (5), (1) University of Florida, Gainesville, FL, USA; (2) Auburn University, Auburn, AL, USA; (3) University of Central Florida, Orlando, FL, USA; (4) Connecticut Agric Exp Station, New Haven, CT, USA; (5) North Florida Research & Education Center, University of Florida, Quincy, FL, USA; (6) Gulf Coast Research and Education Center, University of Florida, Wimauma, FL, USA

Bacterial spot of tomato, incited by four *Xanthomonas* spp., is a devastating bacterial disease worldwide. There have been limited effective chemical controls in Florida following the appearance of copper (Cu)-tolerant strains, which rendered Cu bactericides ineffective. Due to ecological risks, the EPA has proposed that Cu applications should be limited. Therefore, finding effective alternatives to Cu is critical. Our prior research has focused on novel antibacterial properties of 20nm magnesium oxide nanoparticles (nano MgO) against Cu-tolerant *X. perforans* (Xp). Nano MgO and micron sized MgO (0.3 and 0.6 µm) have bactericidal activity against Xp *in vitro*, but only nano MgO has been effective in bacterial spot disease management under field conditions. Based on these findings, in our current study, formulated Mg nanomaterials (i.e., SgMc, Mg-Cu, MgDB) significantly increased antibacterial efficacy when compared to nano MgO *in vitro*. In greenhouse studies, SgMc when applied at 100 µg/mL reduced disease severity compared to the grower's standard, Cu- ethylene-bis-dithiocarbamate (Cu-EDBC), and the water control (UT) (p = 0.05). In the fall 2019 field experiment, Mg-Cu and MgDB at 500 µg/mL significantly reduced disease severity compared to UT whereas Cu-EDBC was not significantly different from UT (p = 0.05). Additional field studies would have to be undertaken to determine consistent efficacy of formulated Mg nanomaterials across multiple production seasons.

Investigating appressorium form and function using proximity-dependent proteomics

N. DULAL (1), R. Proko (2), M. Egan (1), (1) University of Arkansas, Fayetteville, AR, USA; (2) University of Arkansas, Fayetteville, AR, USA

Rice blast disease, caused by the fungus *Magnaporthe oryzae*, destroys enough rice each year to feed 60 million people. To establish disease, *M. oryzae* forms a specialized infection structure called an appressorium which it uses to mechanically break into rice leaves. Essential for this process is the timely formation of a septin ring at the base of the appressorium. Septins are a conserved family of GTP-binding proteins, forming hetero-oligomeric rods and filaments that are assembled into higher-order structures. The *M. oryzae* septin ring organizes the actin cytoskeleton and acts as a diffusion barrier to organize membrane-associated proteins required for appressorium re-polarization and plant infection. Relatively little is known about how higher-order septin structures form in the right place and time to drive infection. We are using a proximity-dependent proteomics approach to identify novel proteins involved in septin ring formation. We genetically tagged the septin protein Sep3 with TurboID and generated a list of putative septin-interacting and proximal proteins. We are using a cell biological and reverse genetic approach to validate and functionally characterize these proteins. Here we report our progress in discovering novel proteins involved in septin and cytoskeleton organization during appressorium morphogenesis. The outcomes of this research will provide new insight into the cellular control of septin organization in a global cereal killer.

Development of leaf spots and fruiting bodies by *Stagonosporopsis citrulli* on fungicide-treated watermelon leaves in the field

A. P. KEINATH, Coastal Research and Education Center, Clemson University, Charleston, SC, USA

Fungicide applications are the main method to manage gummy stem blight on watermelon and other cucurbits, although fungicides do not completely suppress formation of leaf spots. It is not known if fungicides affect leaf spot expansion or development of fruiting bodies by *Stagonosporopsis citrulli*. The fungicides Switch (cyprodinil + fludioxonil), Inspire Super (cyprodinil + difenoconazole), Vanguard (cyprodinil), Cannonball (fludioxonil), and Inspire (difenoconazole) were applied to watermelon in rotation with Bravo (chlorothalonil) in fall 2017, 2018, and 2019. Water and Bravo served as control treatments. All fungicides reduced disease severity (percentage of leaf area diseased) and AUDPC in field plots compared to water (non-significant year-by-treatment interaction). Switch and Inspire Super rotated with Bravo reduced disease severity and AUDPC more than Bravo applied alone. Fungicides did not affect the number, diameter, or area of individual, older leaf spots. All fungicides reduced the number of leaf spots with fruiting bodies of *S. citrulli* compared to water ($P < 0.05$). Switch and Inspire Super rotated with Bravo reduced the percentage of leaf spots with fruiting bodies, and the diameter and area of the portions of leaf spots covered with fruiting bodies, compared to water and to Bravo applied alone. Premix fungicides containing cyprodinil reduced fruiting body formation by *S. citrulli*, which may partially explain their efficacy in managing gummy stem blight.

Comparative performance of fungicides and biorational products in managing post-harvest *Botrytis cinerea* infection on hydrangea cut flowers

R. BIKI (1), C. L. Palmer (2), F. Baysal-Gurel (1), (1) Tennessee State University, McMinnville, TN, USA; (2) IR-4, Princeton, NJ, USA

Botrytis cinerea is the most problematic and notorious post-harvest pathogen of the cut flower industry. It causes flower blight, leaf blight and stem rot, reducing the ornamental value (longevity, color, texture, etc.) of flowers, ultimately making them unsalable. The objective of this study was to identify effective foliar and dip applications of fungicides and biorational products for *B. cinerea* management on cut flowers that can be easily and readily adopted by growers. In the foliar application, hydrangea plants were sprayed with treatment solution 3 days before harvesting flowers. In the dip application, hydrangea cut flowers were dipped in treatment solution after harvest. Once the treatment dried, *B. cinerea* spore suspensions were used for inoculation, excluding the control. Flowers were stored in cold storage for 3 days and then displayed under simulated retail conditions; then disease severity and post-harvest shelf life was evaluated. All treatments reduced disease progress (AUDPC) in both application methods, but the treatments that significantly lowered AUDPC were Astun, Broadfoam, Orkestra and Medallion in dip application methods whereas Astun and Orkestra as foliar applications were similar to the non-treated, inoculated control. These treatments effectively maintained longer vase life compared to the non-treated, inoculated control. Information from this research can assist growers in post-harvest Botrytis blight management in cut flowers.

Exploring new control methods for '*Candidatus Liberibacter asiaticus*' by using genetically engineered *Liberibacter crescens* strains

M. SENA VELEZ (1), J. Portilla (1), S. Holland (1), M. Jain (2), D. W. Gabriel (2), K. M. M. Jones (1), (1) Florida State University, Tallahassee, FL, USA; (2) University of Florida, Gainesville, FL, USA

'*Candidatus Liberibacter asiaticus*' (CLAs) is the causal agent of the devastating Huanglongbing/citrus greening disease. Some antibiotics are effective against CLAs, but there is great need for additional control methods. The main constraint in the study of CLAs is its unculturability. We are using *Liberibacter crescens* (*Lcr*), the only culturable member of the genus, as a surrogate for testing anti-CLAs control methods. We have produced *Lcr* strains that are more 'CLAs-like' by expressing outer membrane protein A from CLAs in *Lcr*. Our rationale for producing *Lcr*^{CLAs-OmpA} strains was to serve as 'bacteriophage trap' strains, since the ortholog of CLAs-OmpA serves as a bacteriophage receptor in related bacteria. We continue to use these strains in phage screening, but we are also testing the

relative sensitivity to antimicrobial compounds of these *Lcr*^{CLas-OmpA} strains with altered outer membrane properties. Antimicrobial peptides (AMPs) serve as components of innate immunity in many organisms. AMPs kill pathogens by diverse mechanisms, including membrane disruption, and are good candidates for the control of bacterial diseases. Three AMPs, Apidaecin IB, Cecropin B and Melittin, were tested for growth inhibition of *Lcr*^{CLas-OmpA} compared with control strains. The strongest effects were seen with Melittin. These studies demonstrate the utility of *Lcr* as a model for testing anti-CLas control methods.

Pro-viral function of the glycolytic enzyme FBA1 during TBSV replication

M. MOLHO, P. D. Nagy, University of Kentucky, Lexington, KY, USA

Plus-stranded (+) RNA viruses are the largest family of viruses that infect plants, causing important economic losses in different crops. Tomato bushy stunt virus (TBSV), a small positive-stranded RNA virus, has emerged as a model virus to study host-virus interactions. TBSV encodes for only five proteins, therefore, to infect the host cell TBSV co-opts selected host components and subvert specific molecular pathways. TBSV also recruits the glycolytic enzymes, PGK1 and PK, to supply the energy required at the replication site. To further study if TBSV recruits other glycolytic enzymes and possibly the whole glycolytic pathway, we performed a proteomic screening using Arabidopsis proteins. We found that TBSV viral replication proteins interact with 88 host proteins, including Fructose 1,6 biphosphate aldolase (FBA1), a key enzyme in plants involved in glycolysis and glucogenesis. Purification assays determined that TBSV directly recruits FBA1 to the site of replication. Furthermore, the down-regulation of FBA1 in *N. benthamiana* inhibits TBSV replication and reduces ATP levels at the viral replication site.

Overall, we have discovered that the local production of ATP by the co-opted glycolytic enzymes within the viral replication organelle is required for efficient TBSV replication. Further studies need to be done to address the potential moonlighting function of FBA1 in TBSV replication.

Evaluating the relative contribution of fungicides applied alone or as mixtures for management of *Venturia effusa* in commercial pecan orchards

L. C. MOORE, T. B. Breneman, K. L. Stevenson, University of Georgia, Tifton, GA, USA

Venturia effusa causes pecan scab and can be resistant to several fungicide classes. In 2019, fungicides were applied repeatedly at 14-day intervals to replicated single fruiting terminals of pecan trees in 10 orchards in Georgia. These fungicides included labeled rates of tebuconazole (TE), difenoconazole (DI), mefentrifluconazole (ME), azoxystrobin (AZ), (DI+AZ), pydiflumetofen (PY), (PY + DI), microthiol sulfur (S), (S+ME), and a nontreated control. Single fungicides were used at the rate found in mixtures. The mean relative growth of *V. effusa* from these orchards on media amended with 3 ppm TE was 63%, and TE alone gave only 34% control of scab. The other DMI's (DI and ME) each gave 76% control. The DI + AZ mix gave 78% control, similar to the DI alone, indicating that DI provides most of the activity. AZ alone gave 54% control. Adding S to ME actually decreased its efficacy by 21% compared to ME alone, contradicting results found in other pathosystems. PY is an SDHI fungicide, a class not previously used on pecans. It gave excellent control at all locations (94%), similar to the control with PY + DI (97%). These results give insight into which active ingredients are most important in premixes, as well as helps optimize use patterns for effective fungicide resistance management.

Defining dispersal mechanisms in storage for *Ceratocystis fimbriata*, causal agent of black rot in sweetpotatoes

M. STAHR (1), M. Bertone (2), A. Huseh (2), L. M. Quesada (3), (1) NCSU, Raleigh, NC, USA; (2) North Carolina State University, Raleigh, NC, USA; (3) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA

Sweetpotato black rot, caused by *Ceratocystis fimbriata*, is frequently seen in packing and storage facilities, but little is known as to how pathogen dispersal occurs. Thus, this study was designed to investigate i) potential insect vectors and ii) dump tank water as means of dispersal. A population of *Drosophila hydei* was reared in a controlled environment, and adult flies were exposed to healthy or *C. fimbriata*-infected roots for 96-hrs. Ten flies were recovered from both treatments and externally washed with, or surface sterilized and crushed into, a 5% tween solution. All solutions were plated onto amended carrot agar and checked for *C. fimbriata* colony growth for up to 1 week. Recovery of *C. fimbriata* was low, but almost exclusively found in internal washes of flies exposed to infected roots. Next, *C. fimbriata* spore suspensions were diluted (0, 5, 50, 500, & 5000 spores/mL), aged (0, 24, 48, 96, & 144 hrs), or exposed to four commercial sanitizers to test the effect of inoculum density, age, and sanitizer presence on water dispersal. Roots were soaked in the different suspensions for 20-min, stored at 29°C for a 14-day period, and rated for incidence every other day. Disease incidence significantly decreased as inoculum concentration decreased, inoculum age increased, and in the presence of chlorine-based sanitizers. These findings indicate that *D. hydei* and improperly managed dump tanks could be aiding in pathogen dispersal, but further research is still needed.

Temperature effect on *Fusarium oxysporum* f. sp. *niveum* disease incidence in watermelon seedlings

Z. XU, N. S. Dufault, University of Florida, Gainesville, FL, USA

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* (Fon), is a critical watermelon (*Citrullus lanatus*) disease in Florida and the southeast US, which can cause yield losses near 100% when severe. Previous field research has shown that temperatures above 30°C

can reduce *Fusarium* wilt epidemics by 50%. An improved understanding of temperature effects on Fon watermelon infections is vital to assessing disease risk and selecting proper management techniques. It was hypothesized that *Fusarium* wilt would not be present at temperatures above 30°C. The study objective is to evaluate *Fusarium* wilt disease incidence in response to varying temperatures. Three Fon races (race 1, race 2 and race 3) from five isolates were inoculated on to two watermelon cultivars (Black Diamond and Melody) that were kept at four different temperatures (22°C, 26°C, 30°C and 34°C). Results showed that disease incidence was highest (53% Melody, 78% Black Diamond) at 26°C, reduced at 30°C (33% Melody, 43% Black Diamond), and limited (0% for both cultivars) at 22°C and 34°C. The three races had similar disease incidence with temperature trends across the cultivars. It is apparent from these results that Fon infections, for the five isolates tested, are limited when the temperature is less than or equal to 22°C or higher than or equal to 34°C. Thus, management strategies (e.g., delaying planting) can be modified to take advantage of environments when soil temperatures reach these thresholds.

Two season evaluation of diversity, pathogenicity and mycotoxin production potential of *Fusarium* spp. in North Carolina

S. M. COCHRAN (1), B. Bronico (2), M. Salazar (3), L. D. Thiessen (3), (1) Raleigh, NC, USA; (2) NCSU, NC, USA; (3) North Carolina State University, Raleigh, NC, USA

Corn ear rot is caused by several fungi including *Fusarium* and *Aspergillus* spp. that can produce mycotoxins, reduce yield, and decrease profit for growers. North Carolina's warm, humid climate makes the environment favorable for corn ear rot outbreaks during the growing season. Corn ear rot samples were collected throughout North Carolina for two consecutive growing seasons, and numerous species of *Fusarium* spp. and *Aspergillus* spp. have been identified from these samples. Isolates were molecularly identified with Sanger Sequencing using the ITS, EF, RPB2, and TUB2 gene regions. The isolated *Fusarium* spp. were evaluated for potential deoxynivalenol, fumonisin, and zearalenone mycotoxin production through PCR and gel electrophoresis with the primers Fum1, Tri6, Tri7, Fum13, Zen, Tri13, FCU, Tri5, and Fum21. The pathogenicity of *Fusarium* spp. isolates were evaluated through a detached leaf assay and were statistically analyzed for significance. The data collected from the past two seasons will be used to inform North Carolina corn growers of the diversity of *Fusarium* spp. across the state. The pathogenicity of these species and mycotoxin production potential will be presented in attempts to develop a successful integrated pest management program for this disease.

Predicting the *Peronospora belbahrii* secretome for *in silico* identification of effector proteins

J. R. STANDISH (1), S. Purayannur (1), M. Bowman (2), K. L. Childs (2), M. Tian (3), L. M. Quesada (1), (1) Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, USA; (2) Department of Plant Biology, Michigan State University, East Lansing, MI, USA; (3) University of Hawaii at Manoa, Honolulu, HI, USA

Peronospora belbahrii is an oomycete and the cause of basil downy mildew, a disease affecting basil production worldwide. As an obligate biotroph, *P. belbahrii* is dependent on a living host to obtain nutrients for survival. Many oomycete plant pathogens secrete apoplastic and cytoplasmic effector proteins to target host processes, allowing for their colonization of host tissue. The secretome and effector catalog for *P. belbahrii* were predicted based on an Illumina draft genome assembly. Overall, 1,282 proteins possessed a signal peptide and no transmembrane domain, and among those proteins, 230 were predicted as probable apoplastic effectors. A total of 57 cytoplasmic effectors were predicted with various motifs, including the RxLR-EER (31), RxLR (11), EER (5), GxLR-EER (3), QxLR-EER (2), RxLK-EER (2), KxLR-EER (1), HxLR-EER (1), and a single CRN protein with the LFLAK-HVLV motif. RNAseq data from four additional isolates were used to determine transcript presence/absence variation. Of the 57 cytoplasmic effectors, all but three were expressed in all five isolates. A QxLR-EER was observed only in the reference isolate; a RxLR-EER was observed in the reference and one other isolate; and an RxLR was observed in the reference and all but one other isolate. The conserved expression of these cytoplasmic effectors across several isolates makes them ideal targets for identifying corresponding R genes in breeding efforts where disease resistance is a priority.

Pan-genome-wide analysis of *Pantoea ananatis* identified genes associated with onion pathogenicity

G. AGARWAL (1), D. Choudhary (1), S. Stice (2), B. H. Kvitko (2), R. D. Gitaitis (1), B. Dutta (1), (1) University of Georgia, Tifton, GA, USA; (2) University of Georgia, Athens, GA, USA

Pantoea ananatis is a member of a *Pantoea* spp. complex that causes center rot of onion, which significantly affects onion yield and quality. The bacterium lacks type II or type III or type IV secretion systems but requires a proposed biosynthetic gene-cluster, HiVir, for a phosphonate secondary metabolite (located in chromosome) and the onion-virulence regions, OVR, (localized on a megaplasmid) for onion pathogenicity and virulence. However, pathogenicity and virulence factors other than already known need further investigation. We conducted a pan-genome wide analysis (pan-GWAS) with *P. ananatis* strains (n = 81) possessing varying levels of aggressiveness based on red onion scale clearing. Our phylogenomic analysis using the presence and absence variants (PAVs) could distinguish aggressive vs. non-pathogenic *P. ananatis* strains based on the presence or absence of the HiVir cluster. A pan-GWAS conducted using PAVs and phenotyping data identified potential candidate genes associated with pathogenicity in onions. Annotated functions of these genes included leucine biosynthesis, methyltransferases, monooxygenases, phosphonopyruvate hydrolase and l-amino-acid ligase. Pan-genomic differences not only predicted genes associated with pathogenicity and differentiated pathogenic from non-pathogenic strains but also provided potential evidence of constantly evolving accessory genes in this bacterium.

Citrus greening disease in Georgia citrus

T. L. STACKHOUSE (1), S. Waliullah (2), J. Price (3), J. E. Oliver (4), E. Ali (1), (1) University of Georgia, Tifton, GA, USA; (2) University of Georgia, GA, USA; (3) University of Georgia, Valdosta, GA, USA; (4) Department of Plant Pathology, University of Georgia, Tifton, GA, USA

Citrus greening disease, also known as huanglongbing (HLB), is the most serious disease of citrus worldwide. This disease, caused by the bacterium *Candidatus Liberibacter asiaticus* (CLAs) and spread by the Asian citrus psyllid, leads to lower fruit quality and reduced tree productivity. Commercial planting of citrus trees in Georgia has increased exponentially in recent years, with plantings in at least 32 counties. Georgia has been quarantined for HLB since 2009, following confirmation of the disease in a residential citrus tree in Savannah, Georgia in 2008. Surveys of Georgia citrus have not been previously conducted, so little is known about the statewide CLAs distribution. To better understand CLAs distribution in Georgia citrus, a survey was conducted of commercial and residential citrus trees within coastal and southern Georgia. In total, 94 samples from 11 counties were collected and screened for CLAs via molecular testing. Results revealed the presence of CLAs in 9% of the tested samples overall, and in three counties where HLB had not been previously reported. These findings confirmed for the first time that HLB is present in inland southern Georgia, and suggest that in addition to establishing their orchards with HLB-free citrus stock, commercial citrus growers in this region should vigilantly monitor new plantings. Infected commercial and residential citrus trees should be removed promptly to prevent further disease spread within southern Georgia.

Does crop rotation influence inoculum density of *Xylaria* sp., the pathogen of taproot decline of soybean?

A. BADIAL (1), K. Phillips (1), T. Wilkerson (2), M. Tomaso-Peterson (3), (1) Mississippi State University, Starkville, MS, USA; (2) MSU Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Stoneville, MS, USA; (3) Mississippi State University, Mississippi State, MS, USA

Taproot decline, caused by *Xylaria* sp., is a prominent disease of soybean in Mississippi. Previous research has shown that *Xylaria* sp. is not host-specific to soybean but readily infects other important agronomic crops. This result is concerning as crop rotation and double-cropping are important practices for improving soil health and disease management in soybean. This study focuses on determining the effect continuous soybean and rotational crops have on the inoculum density of *Xylaria* sp. in the soil. Greenhouse studies were established to resemble a four-rotation planting cycle (12-wk) using corn, cotton, and wheat each in rotation with soybean. A *Xylaria* sp. primer was developed and a standard curve determined to quantify inoculum density based on the number of DNA copies. The soil was infested with *Xylaria* sp. prior to planting the initial rotation. Soil cores were removed 7-d post inoculation/planting to establish a baseline quantity of inoculum. At 12-wk, plants were removed at the soil line leaving the root material intact as substrate for *Xylaria* sp. Soil samples were taken and the second rotation was planted. DNA was extracted from 12-wk soil and prepared for PCR amplification. The baseline quantity of *Xylaria* sp. DNA in the soil at 7-d was 19,864 copies. Following the first rotation cycle, inoculum density increased 21%. Up-to-date results following the second rotation planting cycle will also be presented.

Report of the Cottonseed Treatment Committee for 2019

R. GUYER (1), S. Pate (2), T. Allen (3), P. D. Colyer (4), T. S. Isakeit (5), R. Kemeraite (6), K. S. Lawrence (7), H. L. Mehl (8), P. Price (9), A. Rojas (10), L. D. Thiessen (11), T. Wilkerson (12), H. M. Kelly (1), (1) West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA; (2) University of Tennessee, Jackson, TN, USA; (3) Delta Research and Extension Center, Mississippi State University, Stoneville, MS, USA; (4) Louisiana State Univ Agric Center, Bossier City, LA, USA; (5) Dept of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA; (6) University of Georgia, Tifton, GA, USA; (7) Entomology and Plant Pathology, Auburn University, Auburn, AL, USA; (8) Virginia Tech Tidewater AREC, Suffolk, VA, USA; (9) LSU AgCenter, Macon Ridge Research Station, Winnsboro, LA, USA; (10) University of Arkansas, Fayetteville, AR, USA; (11) North Carolina State University, Raleigh, NC, USA; (12) MSU Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Stoneville, MS, USA

The 2019 National Cottonseed Treatment Program evaluated cotton seedling survival for a number of fungicide seed treatment combinations over diverse environmental conditions and populations of cotton seedling pathogens. Four control combinations targeted pathogens of interest (*Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, and *Thielaviopsis basicola*) and 11 fungicide seed treatment combinations were nominated by chemical industry representatives for evaluation. A total of 12 replicated trials were set up across 9 participating states within the cotton beltwide. Cotton seedlings and soil were screened for presence of 4 seedling pathogens. Detection methods of pathogens included selective media plating, soil baiting, and enzyme-linked immunosorbent assay. Significance of treatments tested, pathogens detected, and pathogen screening methods will be presented.

Impact of the corn Bt trait on mycotoxin levels

E. ZUCHELLI, R. Guyer, H. M. Kelly, S. Stewart, West Tennessee AgResearch and Education Center, University of Tennessee, Jackson, TN, USA

Occurrence of *Aspergillus flavus* and *Fusarium* sp. in corn can lead to the development of mycotoxins such as Aflatoxin (AFL) and Fumonisin (FMS), respectively. Occurrence of both pathogens are related to insect damage due to wounds which provide infection pathways. Both AFL and FMS are toxic secondary metabolites capable of causing disease and death in both humans and animals.

Disease management includes avoiding plant stress by managing sowing date, and avoiding insect damages by using Bt traits. This research evaluated the impact of different Bt traits (Cry protein and Vip protein hybrids) compared to non-Bt, plant date (April vs. May), and location on AFL and FMS production in corn. Sister lines of Dekalb DKC66-94 and Pioneer P1637 were planted in a randomized complete block design and four replicates. The Pioneer P1637 hybrid was also planted at two locations and two planting dates. Results show no difference between locations or planting dates for AFL and FMS levels. Only in the P1637 hybrid, both Cry and Vip traits reduced FMS compared to non-Bt, although only the Vip trait numerically reduced FMS values below FDA guidance levels. Further results on mycotoxin levels and insect damage will also be discussed.

Turfgrass management practices influence fungicide fate on golf course putting greens

C. STEPHENS, T. Gannon, J. P. Kerns, North Carolina State University, Raleigh, NC, USA

Irrigation is typically applied immediately following fungicide applications when targeting root diseases on golf course putting greens. However, the fate of fungicides following post-application mowing and irrigation is not well documented. The objective of this study was to determine the influence of post-application mowing and irrigation timing on fungicide residue in turfgrass clippings and the soil profile. Single fungicide applications of pyraclostrobin, triadimefon, and penthiopyrad were applied and 0.64 cm of post-application irrigation was applied either immediately or 6 hours following fungicide application. Clippings were collected at 0, 1, or 3 days after treatment (DAT). Soil cores were collected 0, 1, 3, 5, 7, and 14 DAT and dissected into remaining above ground vegetation (RAV), 0–2.54 cm, 2.54–5.08 cm, and 5.08–7.62 cm depths. Samples were homogenized and fungicide residue was analyzed using liquid chromatography mass spectrophotometry. Fungicide recovery as percent of applied ranged from 90–93%, 92–99%, and 92–95% at 0 DAT for pyraclostrobin, triadimefon, and penthiopyrad, respectively. A minor amount of fungicide (0.19–2.31%) was removed with turfgrass clippings regardless of mowing and irrigation timing. Fungicide was only detected in the 5.08 to 7.62-cm depth at 14 DAT when irrigated immediately. Irrigating immediately generally resulted in greater fungicide movement into deeper soil depths.

Detection of airborne inoculum of *Nothopassalora personata* in peanut fields using impaction spore traps coupled with a qPCR assay

M. MUNIR, H. Wang, D. J. Anco, Clemson University, Blackville, SC, USA

Late leaf spot (LLS), caused by *Nothopassalora personata*, is the most damaging fungal foliar disease of peanut in the southeastern U.S. As fungicide applications are an important tool in managing LLS, early detection of low levels of inoculum before development of symptoms can help optimize fungicide application timings and improve disease management. We demonstrated the utilization of spore traps coupled with a qPCR assay for detection and quantification of airborne inoculum of *N. personata* in peanut fields. In the 2019 growing season, 2 modified Rotorod style spore traps were placed in 2 peanut fields with a history of LLS. Sampling units were replaced every 2 to 4 days and tested with the qPCR assay developed for *N. personata*, and plots were monitored for symptom development. Number of *N. personata* spores collected on each trap per sampling duration was estimated by comparing C_t values to the assay standard curve and compared to collected weather data. The trapping system was able to detect inoculum as early as 35 to 56 days before visual symptoms developed in the field. While detection varied between plots, results of the trapping system appeared to be related to environmental conditions affecting pathogen life cycle, availability of primary inoculum sources, plot management, and disease development in each plot. This study demonstrated the potential of qPCR-spore trap combination to be used as a decision support tool to assist LLS management.

Environmental bacteria as potent biocontrol agents for controlling *Phytophthora* fruit rot on watermelon

J. TAYLOR (1), A. Boroujerdi (1), T. Pikes (1), R. Harris (1), C. S. Kousik (2), M. K. Mandal PhD (1,2), (1) Claflin University, Orangeburg, SC, USA; (2) U.S. Vegetable Laboratory, USDA ARS, Charleston, SC, USA

Phytophthora fruit rot caused by the oomycete *Phytophthora capsici*, is one of the most devastating pathogen affecting watermelon production in most states in the Southeastern US and current management strategies are not effective. The watermelon fruits can become infected at any stage of maturity, either from direct contact with the soil or from splashing rain or irrigation. Fungicides remain an important method to control losses due to *P. capsici*. However, due to high disease pressure in Southeastern states as a result of extreme weather conditions (e.g., flooding, hurricanes), the dose of fungicide application has been increased leading to phytotoxicity to watermelon plants and development of fungicide insensitivity/resistance in *P. capsici* over the years. Since fully restricting the movement of *P. capsici* in fields is often impossible, one of the best alternative sustainable approaches is to prevent phytotoxicity and fungicide insensitivity in *P. capsici*. This can be done with the use of environmental friendly bacterial biocontrol agents, which can suppress pathogen growth both in field as well as storage conditions. We have identified several bacterial isolates from natural environments, which have potential to inhibit *P. capsici* growth on V8 media. Some bacterial isolates suppressed *Phytophthora* fruit rot development on watermelon. Identification of the biomolecules having antifungal activity from five bacterial isolates using NMR and LC-MS/MS are in progress.

Evaluation of succinate dehydrogenase inhibitors for control of southern root-knot nematode and fungal pathogens in watermelon

T. S. WONG, L. Quesada-Ocampo, North Carolina State University, Raleigh, NC, USA

Newly developed succinate dehydrogenase inhibitors (SDHIs) with fungicide and potential nematicide activity provide the opportunity to control multiple diseases with one compound. However, the use of SDHIs poses a high risk of fungal pathogen populations developing resistance. If newer SDHIs were to be used to control nematodes, its potential effect on fungal population should be examined. In this study, we aimed to determine the sensitivity of *Meloidogyne incognita* race 4 (MI4), *Fusarium oxysporum* f. sp. *niveum* (FON), and *Stagonosporopsis citrulli* (SCIT), to existing and new SDHIs: benzovindiflupyr, fluopyram, an experimental, and pydiflumetofen. Assays targeting two life stages were conducted for FON and MI4. Cross-sensitivity for SDHIs were also determined for the fungal pathogens. Findings revealed that all SDHIs had fungicidal activity against 19 SCIT isolates and were most sensitive to pydiflumetofen. For FON, we found isolates to be most sensitive to pydiflumetofen and the experimental in the conidial germination assay. In addition, positive correlations between pydiflumetofen to other SDHIs were observed, which indicate a potential for cross-resistance between these SDHIs. Only fluopyram and the experimental were effective against MI4, particularly in the egg hatch assay. Overall, results suggest that the experimental may be used for managing root-knot nematode, whereas it should be used judiciously for *Fusarium* wilt of watermelon and gummy stem blight.

Evaluation of fungicides and cultivars for control of cucumber downy mildew

M. ADAMS, K. D'Arcangelo, L. Quesada-Ocampo, North Carolina State University, Raleigh, NC, USA

Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is the most economically important disease of cucumber in the United States (US). The re-emergence of *P. cubensis* in the US in 2004 resulted in a failure of previously effective cucumber host resistance and the pathogen has become insensitive to several fungicides. The implementation of an integrated disease management strategy is recommended to minimize the development of fungicide resistance in pathogen populations but few studies have been conducted to determine the individual effectiveness of these approaches. To determine the contribution of fungicide applications and host tolerance on CDM management and pickling cucumber yield, multi-year field trial experiments were conducted in North Carolina. Evaluations included single-product fungicide applications as well as program treatments on susceptible and tolerant varieties. Our analysis revealed that when compared to the non-treated controls, spray programs, which alternate fungicide modes of action and use tank mixes with protectants, resulted in lower levels of downy mildew, higher marketable cucumber yields and reduced the risk of pathogen populations from overcoming fungicides. In addition, tolerant varieties also displayed lower disease levels than susceptible cucumber varieties.

Introducing, Howler, an innovative, multi-site fungicide

B. MCINNES, AgBiome Innovations, Hahira, GA, USA

AgBiome discovers and develops innovative biological and trait products for crop protection utilizing its proprietary Genesis™ discovery platform. This innovative discovery platform has enabled AgBiome to efficiently capture, fully sequence, and screen the most diverse and unique microbial collection for fungal, nematode, and insect control. Through this process, AgBiome has developed and commercialized Howler fungicide. Howler's novel, multi-site, mode-of-action controls soilborne and foliar diseases to include *Rhizoctonia*, *Botrytis*, downy mildew, powdery mildew, and soil-borne *Phytophthora*.

Pathogenic and genetic diversity of *Corynespora cassiicola* isolates from tomato across Florida

K. V. XAVIER (1), L. G. Sumabat (2), M. T. Brewer (2), G. E. Vallad (3), (1) University of Florida, Wimauma, FL, USA; (2) University of Georgia, Athens, GA, USA; (3) Gulf Coast Research and Education Center, University of Florida, Wimauma, FL, USA

Target spot, caused by *Corynespora cassiicola* (Berk. & Curt) Wei, is an economically important disease on tomato in Florida. The genetic diversity among *C. cassiicola* isolates collected from Florida tomatoes and other hosts was characterized. Multilocus sequence analysis of 16 representative isolates of *C. cassiicola* based on rDNA ITS, *caa5*, *ga4*, and *act1* determined that most tomato isolates grouped into phylogenetic lineage 4, while isolates from cotton, sesame and cucumber, along with one tomato isolate, grouped into phylogenetic lineage 1. The genetic diversity of 127 *C. cassiicola* isolates from tomato and other hosts was further separated into 33 haplotypes based on eight microsatellite markers. Isolates were genetically distinct based on host species. One hundred twenty-one tomato isolates were separated into 29 haplotypes. Ten representative isolates of *C. cassiicola* from tomato were further evaluated for aggressiveness on fruit and seedlings of tomato cv. FL47. Most of the isolates caused intermediate to high levels of disease when inoculated onto seedlings, while aggressiveness varied when inoculated onto ripe fruit. This high level of pathogenic and genetic variability among isolates from tomato and other hosts might complicate current breeding efforts to develop resistant tomato cultivars.

Hold on to your roots: Bacterial maintenance on plant roots

N. ECKSHAIN-LEVI, S. Harris, E. Shank, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

To address food security, agricultural yields must increase to match the growing human population. Currently, there is a strong push to develop more sustainable agricultural practices, one of which is using plant-growth-promoting bacteria (PGPB). Those PGPB are marketed as being able to improve plant yields, help crops to cope with harsh environmental conditions, and suppress the growth of plant pathogens and decrease disease symptoms. One concern of using PGPB-based interventions is that some of these bacteria don't

maintain original levels of colonization on plant roots, therefore reducing their ability to positively affect plants over time. We used *Arabidopsis thaliana* seedlings grown in a hydroponic system to screen for bacterial colonization and maintenance. Our method is a fast, easy-to-adapt, high-throughput system. We characterized the ability of 96 fully genome-sequenced bacterial strains isolated from the *A. thaliana* rhizosphere to colonize and maintain on *Arabidopsis* roots. Using this process, we also identified three bacterial strains that were able to significantly improve the maintenance of *Bacillus subtilis* (a bacterium that typically does not maintain well on its own). Our long-term goal is to identify functional microbial consortia that can associate with plant roots consistently over time and thus improve plant health and yields.

Bacterial blight of cotton: Attributes of the causal agent (*Xanthomonas citri* subsp. *malvacearum*) and impact of management on disease

T. A. WHEELER (1), T. W. Allen Jr. (2), R. Bart (3), T. S. Isakeit (4), R. Kemeraït (5), J. E. Woodward (6), (1) Texas A&M AgriLife Research, Lubbock, TX, USA; (2) Mississippi State Univ, Stoneville, MS, USA; (3) Donald Danforth Plant Science Center, St Louis, MO, USA; (4) Dept of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA; (5) University of Georgia, Tifton, GA, USA; (6) Texas A&M AgriLife Extension Service, Lubbock, TX, USA

Bacterial blight of cotton, caused by *Xanthomonas citri* subsp. *malvacearum* (Xcm), reemerged as a serious problem in the U.S. starting in 2011 in Arkansas and Mississippi. Within the next five years, it became a widespread problem over much of the U.S. cotton acreage. Questions regarding the resurgence of this disease centered around the causal agent. While Xcm is characterized by race, which was historically determined by pathogenicity testing on many different and obsolete cotton lines, a more modern concept of pathogen characterization can be based on virulence factors, in this case type 3 effectors associated with isolates of Xcm. A series of Xcm isolates from across the U.S. were collected and tested for different type 3 effectors. Isolates from TX were also tested for pathogenicity on a race 18-susceptible variety and on S295, which is resistant to Xcm race 18. In MS, commercial varieties were inoculated with Xcm and evaluated for severity of symptom development. As a current management practice, Extension specialists have emphasized planting disease-resistant varieties. In 2016, before the start of our USDA-NIFA IPM project, cotton producers in GA, MS, and TX planted Xcm-susceptible cotton varieties on 90, 77, and 66% of their land, respectively. By 2019, cotton producers in these states were planting Xcm-susceptible varieties on 32, 11, and 25% of their land, respectively. By 2019, bacterial blight had become mostly an inconsequential disease in these states.

Virginia IPM: Ensuring food security, public health, and pollinator protection

D. B. LANGSTON JR. (1), E. A. Bush (2), J. Derr (3), M. Flessner (2), C. Laub (4), H. L. Mehl (1), D. Miller (5), D. G. Pfeiffer (6), S. Taylor (7), M. J. Weaver (2), J. Wilson (2), (1) Virginia Tech Tidewater AREC, Suffolk, VA, USA; (2) Virginia Tech, Blacksburg, VA, USA; (3) Weed Science Society of America, Virginia Beach, VA, USA; (4) Virginia Tech Entomology Dept., Blacksburg, VA, USA; (5) Virginia tech, Blacksburg, VA, USA; (6) VPI & State University, Blacksburg, VA, USA; (7) Virginia Tech, Suffolk, VA, USA

In Virginia, damage by a diverse and ever-changing array of insects, diseases, and weeds results in significant crop and commodity loss. Other pests pose risks to humans in public housing. Growers, foresters, nurserymen, homeowners, and commercial applicators rely heavily on pesticides. However, these pesticides may have negative impacts on non-target organisms, and pollinator protection is of increasing importance. The overall objectives of Virginia IPM are: 1) to educate clientele from all public sectors in the economic, health, and environmental benefits of transitioning to proven IPM practices; 2) to improve adoption and implementation of higher level IPM practices; and 3) to develop and extend strategies that minimize or enhance safety of chemical inputs while reducing commodity loss, human health risks, and negative impacts on managed and non-managed pollinators. Specific activities conducted to achieve these objectives include identifying and monitoring for new and emerging insect, disease, and weed pests; monitoring pesticide efficacy and resistance; developing and deploying advisories to control diseases and insects; and educating the public about pesticides and pests through electronic media, traditional physical media, and face-to-face experiences.

Development of an IPM Program for Rose rosette disease using systemic acquired resistance inducer and biocontrol agents

M. L. PARET (1), G. Knox (2), X. Martini (1), S. Joseph (3), J. L. Williams-Woodward (4), A. Fife (5), A. Monterrosa (3), F. B. Iriarte (6), C. Alexander (3), S. Da Silva (7), B. Riddle (2), K. Thomas (5), F. Ochoa Corona (8), (1) University of Florida, Quincy, FL, USA; (2) North Florida Research and Education Center, University of Florida, Quincy, FL, USA; (3) University of Georgia, GA, USA; (4) Department of Plant Pathology, University of Georgia, Athens, GA, USA; (5) University of Florida, FL, USA; (6) Univ of Florida - IFAS NFREC, Quincy, FL, USA; (7) North Florida Research & Education Center, University of Florida, Quincy, FL, USA; (8) Oklahoma State University, Stillwater, OK, USA

Rose rosette disease (RRD), caused by *Rose rosette virus* (RRV), is currently the most devastating disease of roses in the U.S. and is vectored by *Phyllocoptes fructiphilus*, an eriophyid mite. During 2018–19, a systemic acquired resistance (SAR) inducer, acibenzolar S-methyl was evaluated on vector populations and RRD in three field experiments in GA. Similarly, rose plant quality was evaluated in two field experiments in rose nurseries in FL and GA. Presence of RRV was tested on samples using RT-qPCR, and Recombinase Polymerase Amplification (RPA). Standardization of Loop-mediated isothermal amplification (LAMP) assay was conducted in OK. A vector survey was conducted in GA and FL. Attraction of phytoseiid mite, *Amblyseius swirskii* to RRD-infected and non-infected rose plants was tested using olfactometer and plant volatiles were analyzed using GC-MS. ASM has potential in reducing vector populations but not RRD severity. Rose plant quality was minimally affected with ASM. In 2019, *P. fructiphilus* was discovered for the first time in FL. *Amblyseius swirskii* was attracted to volatiles of RRV-infected rose but not from non-infected roses. A significant reduction of β -

caryophyllene, methyl salicylate and sesquiterpenes was noted in the infected roses whereas, 3-hexanol or 2-ethyl-2-hexanol increased. Studies evaluating the role of an alternate SAR inducer and predatory mites, and the impact of humidity on *P. fructiphilus* and RRD are in progress.

Rural integrated pest management implementations for Louisiana

G. Reagan (1), G. B. PADGETT (2), (1) Louisiana State University, LA, USA; (2) 8105 Tom Bowman Dr, Alexandria, LA, USA

Louisiana Agriculture Plant Industries are valued at over \$12 billion. The goals of our project are: 1) Monitor and discover diseases, insects, and weeds particularly relative to ipmPIPE needs, 2) Review the changing role of pesticide legislation (required by EPA) on Louisiana agriculture, 3) Revise and update IPM publications, and 4) review of IPM principles for more judicious pesticide use. Mexican rice borer pheromone traps placed near sugarcane and rice were used to identify new populations in three parishes during 2018–19. A way the sugarcane industry is using the insect growth regulator (tebufenozide), showing signs of resistance, is by alternating applications with newer environmentally safe chemicals (Prevathon and Novaluron) with different modes of action. Natural enemies are better able to contribute toward IPM. Kim Pope is working on new pesticide application technologies, particularly for paraquat and dicamba. Studies with the phragmites scale insect (pest of Roseau cane) have involved multi-million dollar efforts by Dr. Rodrigo Diaz, LSU and others from the departments of RNR, Plant Pathology and Biology, two ARS-USDA groups and a hydrologist. Ongoing studies involve interactive biology with Roseau cane and key biocontrol organisms. An IPM advisory committee met with the PI and Co-PI to discuss IPM needs. Eight refereed journal articles and 40 non-refereed papers in entomology and plant pathology were produced.

2019 update for the Clemson University Plant and Pest Diagnostic Clinic

M. WILLIAMSON, Clemson University Plant and Pest Diagnostic Clinic, Pendleton, SC, USA

Boxwood dieback, caused by *Colletotrichum* species, probably *C. theobromicola*, has become rather commonplace in South Carolina (SC). *Buxus* species, along with four species/botanical varieties, were diagnosed with this disease in 2019. Twelve diagnoses were recorded, mainly from landscapes. It was obvious in one situation that plants were infected upon purchase from a large out-of-state nursery. Contrast this situation with diagnoses of boxwood blight, where only 3 incidences were recorded. Macrophomina stem rot of sunpatiens (*Impatiens* hybrid), which was reported in Mississippi in 2016, was detected in SC this summer. Crape myrtle bark scale (*Acanthococcus lagerstroemiae*) was detected in SC for the first time from three samples in the midlands. In at least one case, a large plant was heavily infested, suggesting the insect had been present for some time. In the world of turf, zoysiagrass mite (*Aceria (Eriophyes) zoysiae*) was detected on zoysiagrass. On the noxious weed front, itchgrass (*Rottboellia cochinchinensis*), a Federal noxious weed, has been detected widely in the coastal areas and water hyacinth (*Eichhornia crassipes*) was found twice in the midlands and once in the upstate.

The University of Arkansas Plant Health Clinic

S. E. SMITH (1), K. E. Urrea Romero (2), (1) Univ of Arkansas Cooperative Ext Svc, Fayetteville, AR, USA; (2) University of Arkansas, Fayetteville, AR, USA

The Arkansas Plant Health Clinic received 3362 plant samples from November 1, 2018–November 1, 2019. The highest percentage of samples was the category trees and shrubs with 51.22%, followed by turf with 18.41%, fruit and nut 10.95%, vegetables 8.77%, perennials 4.73%, field crops 2.50%, others comprising 3.61%. There were 1648 nematode samples reported. Highlights included the report of boxwood blight caused by *Calonectria pseudonaviculata* for the first time in Arkansas. This sample came from Pulaski county, located in the middle of the state. The Plant Health Clinic participated in a USDA-APHIS trace-forward for *Phytophthora ramorum*. Sixty-seven samples of Rhododendron, Roses, and other plant species samples were tested for *Phytophthora* spp. using ELISA PathoScreen kit (Agdia). Twenty-five samples tested positive for *Phytophthora* spp. DNA was extracted from these sample and sent to Florida or Michigan for molecular identification. A total of four rhododendron samples tested positive for *Phytophthora ramorum*. Furthermore, the clinic published 30 newsletters with 90 articles describing the most common plant problems seen in the Plant Health Clinic during the 2019 growing season. Some of articles were translated into Spanish, and for the first time 10 newsletters were totally translated into Spanish for our Spanish speaker's customers.

Alabama - Auburn University Plant Diagnostic Lab

K. N. CONNER (1), J. C. Jacobi (2), (1) Auburn University, Alabama Cooperative Extension System, Auburn, AL, USA; (2) Alabama Cooperative Extension System, Birmingham, AL, USA

During 2019, the Auburn University Plant Diagnostic Lab (AU-PDL) processed over 4,000 plant samples testing for *Cotton leafroll dwarf virus* (CLRDV). The virus was identified in cotton from 41 counties across Alabama. CLRDV was also identified in four additional states in the southern US, doubling the total number of states where the virus has been confirmed. Protocols were shared with these states for further testing. The AU-PDL also tested over 300 citrus and psyllid samples for HLB from commercial growers, AL Citrus Clean Plant Network (AL-CCPN) and the Alabama Department of Agriculture and Industries (ADAI) annual citrus survey/continuing delimiting surveys during 2019. The ADAI will define their AL HLB quarantine following these results. Industrial

hemp was grown for the first time in AL during 2019. The most common diseases and insects identified on hemp include *Pythium* damping-off (*Pythium* spp.), southern blight (*Sclerotium rolfsii*), olive leaf spot (*Pseudocercospora cannabina*), hemp leaf spot (*Bipolaris* sp.), Fusarium stem canker (*Fusarium* spp.), corn earworm (*Helicoverpa zea*), yellowstriped armyworm (*Spodoptera ornithogalli*), fire ants (*Solenopsis invicta*), hemp russet mite (*Aculops cannabicola*), and carmine spider mite (*Tetranychus cinnabarinus*).

University of Kentucky Plant Disease Diagnostic Laboratory State Report, SPDN Meeting 2020

J. W. BEALE (1), B. S. Kennedy (2), S. J. Long (1), P. Vincelli (1), (1) University of Kentucky, Lexington, KY, USA; (2) University of Kentucky, Princeton, KY, USA

Major renovations to the western Kentucky facilities of the University of Kentucky Plant Disease Diagnostic Laboratory (UK-PDDL) were completed in late 2019. Limited diagnostic services were provided in a temporary plant examination station during the construction period, but the new state-of-the-art facilities will enhance the diagnostic capacities in the future. New and emerging disease problems diagnosed in the UK-PDDL during the 2019 growing season included several first disease reports on hemp, confirmation of laurel wilt on sassafras, increased incidence of angular leaf spot on tobacco, and continued outbreaks of boxwood blight in landscape plantings. Furthermore, wet conditions in early spring followed by drought in some parts of the state contributed to a wide range of abiotic problems as well as infectious diseases. These and other diagnostic samples become the basis of applied research initiatives at UK, which expands the PDDL's impact from the needs of an individual grower to producers throughout the state and region.

Intensive training in plant problem diagnosis by the NC State Plant Disease and Insect Clinic

B. B. SHEW (1), M. Bertone (2), M. J. Munster (2), S. Butler (2), (1) Department of Entomology and Plant Pathology, NC State University, Raleigh, NC, USA; (2) North Carolina State University, Raleigh, NC, USA

Few county extension agents hired in North Carolina have taken a formal course in plant pathology or entomology, and they consistently list plant problem diagnosis as one of their top training needs. To address this need, the NC State Plant Disease and Insect Clinic (PDIC) organized intensive diagnostic training for county agents in the summers of 2018 and 2019. The workshops featured two days of field training followed by 2.5 days in the laboratory. Prior to the workshops, agents took a pre-test to help identify training needs and attended a webinar covering basic concepts of insect and disease identification. Workshops were targeted regionally each year, with field tours featuring mountain crops (apples, vegetables, ornamentals) in 2018 and Coastal Plain crops (blueberries, corn, hemp, cucurbits) in 2019. In addition to PDIC personnel, 7 extension specialists contributed to the training by selecting sites and leading field tours on their crops of expertise. Laboratory training on the NC State campus focused on diagnostics of the major crops in the target regions. Insect identification, microscopy, agent-friendly diagnostic techniques, abiotic and nutrient disorders, and turf diseases were included. Dissecting microscopes with cameras were purchased for selected attendees. We have trained 43 county agents to date, and expect to train 20 agents from the Piedmont region in 2020. Funds for training and microscopes were provided by NC State Cooperative Extension.

Louisiana Plant Diagnostics Report 2019

R. SINGH, Louisiana State Univ AgCenter, Baton Rouge, LA, USA

The LSU AgCenter Plant Diagnostic Center diagnosed 969 samples during 2019. These included 443 physical, 204 digital, 122 citrus, 34 palm and 19 boxwood samples. Majority of samples (631) submitted by commercial client compared to 134 homeowners. The extension agents and specialists submitted 75% of the samples and 25% submitted directly by clients. Samples consisted of ornamental (55%), fruits (25%), trees (9%), vegetables (8%) and turf-grass (3%). A large number of samples were diagnosed as fungal (36%), followed by bacterial (19%) and abiotic (8%). The pathogen was not detected from 33% of samples submitted for specific pathogen detection. Cultural isolation and microscopy were performed on 186 and 102 samples, respectively. One hundred eight samples were diagnosed using ELISA and 186 diagnosis were confirmed with PCR. Four first new diseases including, *Sclerotinia sclerotiorum* causing tip dieback in blueberry, CLRDV in cotton, *Septoria villarsiae* causing leaf spots on *Nymphoides peltata* and *Colletotrichum higginsianum* causing anthracnose on Arugula were found in 2019. The Diagnostic Center developed a diagnostic *TaqMan* real-time PCR assay for detection and quantification of *C. theobromicola*, causal agent of boxwood dieback. A two-day advanced plant diagnostics training was offered to 12 extension field agents. Additionally, two international visiting scholars received a four-month training in conventional and molecular plant diagnostics methods and techniques.

The guardians of our present and future: Plant diagnosticians safeguarding plant health across the state of Florida

S. BEC (1), R. Gazis (2), M. L. Paret (3), P. D. Roberts (4), N. Peres (5), F. B. Iriarte (6), P. Tian (1), J. Fulton (7), C. Lapaire Harmon (8), (1) University of Florida, Plant Diagnostic Center, Gainesville, FL, USA; (2) University of Florida, Homestead, FL, USA; (3) North Florida Research & Education Center, University of Florida, Quincy, FL, USA; (4) University of Florida, Immokalee, FL, USA; (5) University of Florida - Gulf Coast Research and Education Center, Wimauma, FL, USA; (6) Univ of Florida - IFAS NFREC, Quincy, FL, USA; (7) University of Florida, Gainesville, FL, USA; (8) Univ of Florida PDC, Gainesville, FL, FL, USA

Florida's agriculture sector contributes approximately \$7.467 billion annually to the state's economy with high-value crops ranging from citrus, blueberry, avocado, to vegetables, strawberries, and ornamentals. However, this agricultural diversity is threatened by a diversity of pathogens, so the state's crop protection professionals must remain alert. Built on top of the existing extension resources, state-wide diagnostic infrastructure has been augmented through the USDA-NIFA NPDN cooperative agreement to support crop-wide surveillance and information-sharing. Information is communicated directly to stakeholders, or through cooperation with the state's extension service providing access to cutting edge research. To facilitate this access to stakeholders, the state's diagnostic network is distributed in 5 regional diagnostic labs: Panhandle region (Quincy), North (Gainesville), Central (Balm), Southwest (Immokalee), and South (Homestead). In addition to its regional importance, the Gainesville hub is integral to the country's diagnostic service as the regional hub of the Southern United States' National Plant Diagnostic Network. Consequently, samples are received from across the state, country, and world. Here we report a summary of interesting new results, methods, and outreach contributed by state extension diagnostic professionals.

Recent updates on the University of Georgia Extension Plant Pathology Southern Plant Diagnostic Network Plant Disease Clinics and Nematology Lab

A. JOGI (1), P. M. Brannen (1), J. H. Brock (2), B. Dutta (3), G. B. Jagdale (1), R. Kemerait (3), E. L. Little (1), A. D. Martinez-Espinoza (4), J. E. Oliver (2), J. L. Williams-Woodward (1), (1) Department of Plant Pathology, University of Georgia, Athens, GA, USA; (2) Department of Plant Pathology, University of Georgia, Tifton, GA, USA; (3) University of Georgia, Tifton, GA, USA; (4) Department of Plant Pathology, University of Georgia, Griffin, GA, USA

The Department of Plant Pathology at the University of Georgia maintains two Southern Plant Diagnostic Network Extension Plant Disease Clinics in Athens and Tifton. The clinics use a county extension delivery system to provide diagnostic reports to the residents of Georgia. Our clientele includes growers, retailers, arborists, golf courses, extension educators, researchers, and homeowners. We use a web-based database, called Distance Diagnostics through Digital Imaging (DDDI), to store physical and digital sample information and send reports with diagnoses and recommendations to county offices. We also use DDDI to upload data to the National Plant Diagnostic Network National Repository. Between 2017 and 2018, 3,577 commercial and homeowner physical and digital samples were processed, leading to an estimated 4,837 diagnoses. There is also an Extension Nematology lab in the department and during the same period, they processed 10,600 samples for nematode analysis. They use a web-based database, called Nematode Assay Results System (NARS), to store and disseminate nematode diagnosis results. Our programs are committed to providing accurate, unbiased diagnoses and appropriate recommendations which enable timely and effective management.

New state, county records for insect and mite pests, plant diseases in Tennessee in 2019

A. S. WINDHAM (1), F. Hale (1), D. D. Hensley (2), M. T. Windham (2), B. Kauffman (3), R. Florence (4), (1) Soil, Plant, and Pest Center, Nashville, TN, USA; (2) University of Tennessee, Knoxville, TN, USA; (3) University of Tennessee Extension, Nashville, TN, USA; (4) Soil, Plant and Pest Center, Nashville, TN, USA

The UT Extension, Soil, Plant and Pest Center is the NPDN designated laboratory for Tennessee. In 2019, new state and county records were recorded for plant diseases, insects and mites. *Raffaelea lauricola* the causal pathogen of laurel wilt of sassafras was detected for the first time in Montgomery Co., TN in symptomatic trees and later identified in Cheatham, Dickson, Robertson, Williamson and Hamblen Counties. The ambrosia beetle vector of laurel wilt has not been detected. Crape myrtle bark scale (*Acanthococcus lagerstroemiae*) continues to spread within Tennessee. New observations have been made in Knox, Haywood, Rutherford and Williamson Counties. Asian longhorned tick (*Haemaphysalis longicornis*) has been identified in Roane, Jefferson, Claiborne, Cocke, Putnam, Knox, Union and Sevier Counties. Emerald ash borer (*Agrilus planipennis*) has recently been found in Bradley, Carter, Coffee, Johnson, Lewis, Macon, Meigs, Moore, Overton, Pickett and Sequatchie Counties for a total of 63 counties of 95 in TN. Rose rosette virus is widespread in roses throughout TN. *Phyllocoptes fructiphilus*, is the only known eriophyid mite vector of Rose Rosette (RRV) and is found in many states east of the Rocky Mountains. Two additional eriophyid mites, *Callyntrotus schlehtendali* and *Eriophyes eremus*, were found on roses symptomatic for rose rosette in Knox Co., TN. It is not known if these mites are vectors of RRV, but they may depress populations of *P. fructiphilus*.

Trends and impacts, interesting diagnoses, quality control and new testing in the Virginia Tech Plant Disease Clinic

E. A. BUSH, M. A. Hansen, Virginia Tech, Blacksburg, VA, USA

The number of samples received by the Virginia Tech Plant Disease Clinic (PDC) continued to rise in 2019, with approximately 1,700 samples received and approximately 2,150 diagnoses provided, including several first reports to the National Plant Diagnostic Network national repository. The PDC also experienced the first year of industrial hemp production in Virginia and diagnosed many diseases in the crop. Each year the PDC queries growers via an electronic survey regarding changes in knowledge, behavior and outcomes resulting from the PDC's diagnostic services; the survey results demonstrate a range of positive impacts of the PDC. In recent years the PDC has increased standardization and quality control through the use of the lab document management system in EDDMaps. The Clinic is also implementing several new virus tests.

Texas Plant Disease Diagnostic Laboratory at Texas A&M annual report

K. ONG, V. Ayala, S. A. McBride, Texas A&M AgriLife Extension Service, College Station, TX, USA

The Texas Plant Disease Diagnostic Lab (TPDDL) located in College Station is one of several Texas A&M AgriLife Extension Service plant disease diagnostic labs affiliated with the Department of Plant Pathology & Microbiology at Texas A&M University. This SPDN member lab processes over 2300 samples annually. Diagnostic results from these samples help provide a generalized view of plant health issues affecting Texas and help to guide potential research areas for colleagues within Texas A&M College of Agriculture and Life Sciences. In 2019, TPDDL processed 2360 samples with majority of the samples received between March and October (average of 265 samples/month). 23% of 2019 samples processed were in support of the Texas Department of Agriculture regulatory inspection and survey activities. Samples from commercial landscape and ornamental production segments accounted for 17% and 12% respectively, while 13% of samples received in 2019 were from homeowners. Most notable is that 25% of all samples processed in 2019 were citrus samples where detections of citrus greening and citrus canker resulted in quarantine actions.