2014 Southern Division Meeting Abstracts

Abstracts presented at the APS Southern Division meeting in Dallas, Texas, February 2–3, 2014. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows: Albu, S., Price, P., Doyle, V., and Schneider, R. 2014. Cryptic diversity within *Cercospora kikuchii* on soybean in Louisiana. (Abstr.) Phytopathology 104(Suppl. 2):S2.1. <http://dx.doi.org/10.1094/PHYTO-104-5-S2.1>

Cryptic diversity within *Cercospora kikuchii* on soybean in Louisiana

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Phytopathology 104(Suppl. 2):S2.1

Cercospora kikuchii is considered the causal agent of Cercospora leaf blight (CLB) and purple seed stain (PSS), two economically important diseases prevalent in soybean (*Glycine max*) growing regions of the Gulf South. Identification of *C. kikuchii* is predominantly based on host association, cercosporin production and conidial morphology. However, these characters are unreliable for differentiating closely related species. Cercosporin production is variable among isolates and it can be difficult to induce sporulation *in vitro*. Furthermore, recent molecular phylogenetic studies have shown that multiple cryptic species of *Cercospora* are capable of infecting a single host species. Recent studies evaluating genetic diversity among populations of *C. kikuchii* in Louisiana using microsatellites, RAPD-PCR fingerprinting and vegetative compatibility group pairings suggest there are multiple isolated lineages represented by the sampled isolates. Therefore, the etiology of CLB and PSS remains in question. In this study, we use a multilocus phylogenetic approach to determine if fungal isolates responsible for CLB and PSS are monophyletic. We also address the phylogenetic utility of the internal transcribed spacer (ITS) region, actin (ACT) and histone 3 (H3) genes for evaluating intraspecific relationships among isolates. We collected *C. kikuchii* isolates from symptomatic soybean seeds and leaves in 27 parishes throughout Louisiana during 2000, 2011 and 2012. Multiple haplotypes were observed at each locus, though individual markers varied in their resolving power. There was insufficient phylogenetic signal at ITS to resolve specific or subspecific lineages within the genus *Cercospora*. ACT and H3 provided higher resolution than ITS and *C. kikuchii* was paraphyletic in both analyses. However, node support values were generally low across both topologies indicating the need to develop better phylogenetic markers for *Cercospora*. These results suggest that multiple independent lineages of *C. kikuchii* are present in Louisiana. Alternatively, *C. kikuchii* may be a complex of species capable of infecting soybean.

Distribution of soybean vein necrosis virus (SVNV) in Oklahoma

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Phytopathology 104(Suppl. 2):S2.1

Soybean is one of the important legume crops in Oklahoma which is grown on more than 500,000 acres annually. Recently, a new disease of soybean caused

by Soybean vein necrosis virus (SVNV) has been reported in more than 10 states. No information was available about the presence of SVNV in Oklahoma. During the 2012 growing season, soybean fields were surveyed in 18 major soybean growing counties. Symptomatic leaf samples were tested by dot-immunobinding assay (DIBA) using SVNV antisera. Results showed that the incidence of SVNV ranged from 0–30% in soybean fields mostly in eastern parts of the state. Infection of SVNV was further confirmed by reverse transcription (RT)-PCR using SVNV-specific primers. These results will be discussed in the context of the future threats and possible epidemics caused by SVNV to soybean crops in Oklahoma.

Development of an assay for rapid detection and monitoring of *Verticillium dahliae* in woody plants by real-time polymerase chain reaction (PCR)

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Phytopathology 104(Suppl. 2):S2.1

Verticillium wilt, caused by *Verticillium dahliae* Kleb, is one of the most economically important diseases of woody hosts such as ash, sugar maple, and redbud. The causal agent has a broad host range, including not only woody hosts but also important vegetable and field crops, and it is distributed worldwide. Diagnosis of *V. dahliae* in infected woody hosts is often based on the occurrence of vascular discoloration and time consuming isolation. However, not all woody hosts exhibit vascular discoloration symptoms, and not all vascular discoloration symptoms are due to infection by *V. dahliae*. In this study, two real-time polymerase chain reaction (PCR) assays were optimized and employed for rapid and accurate detection of *V. dahliae* in different woody hosts. High-quality DNA was extracted from infected woody hosts by collecting drill press shavings from sample tissue, bead beating, and extracting using a CTAB method. The PCR assays were based on two previously reported specific primer sets for *V. dahliae* on different hosts (VertBt-F/VertBt-R and VDS1/VDS2). The VertB primers amplified a specific 115-bp fragment, while the VDS primers amplified a specific 540-bp fragment. The PCR-based methods developed here could be used as rapid tools for pathogen detection and monitoring, thus informing plant pathogen management decisions.

From research stations to commercial fields: Survey, detection and identification of sweetpotato viruses in North Carolina

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Phytopathology 104(Suppl. 2):S2.1

Limited information on the status of sweetpotato viruses in North Carolina led us to conduct field surveys in research stations and commercial fields using biological, serological and molecular detection methods. Experiments were carried out at research stations in 2012 and at commercial fields in 2013 growing seasons. Susceptible indicator plants (*Ipomoea batatas*) were evenly distributed in sweetpotato experimental plots at two research stations in NC. Naturally infected indicator plants (n = 129) showing virus-like symptoms

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

<http://dx.doi.org/10.1094/PHYTO-104-5-S2.1>

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were collected and tested for the presence of viruses by NCM-ELISA, multiplex PCR, multiplex and quantitative RT-PCR. Single and mixed viral infections were found in 34% and 66% of the samples, respectively; with potyviruses mixed infections being the most common. *Sweet potato feathery mottle virus* (SPFMV) was the most frequent followed by *Sweet potato virus G* (SPVG), *Sweet potato virus C* (SPVC), *Sweet potato leaf curl virus* (SPLCV), *Sweet potato chlorotic stunt virus* (SPCSV) and *Sweet potato virus 2* (SPV2). Additionally, naturally infected sweetpotato samples (n = 122) were collected in the same experimental plots and they were virus tested using the same procedures. Results showed a similar trend of viral infections as in indicator plants. The same experimental layout for indicator plants was repeated in 2013 at two commercial fields. Most of the indicator plants (n = 135) were symptomless and virus testing data showed potyvirus infections ranging from 0.7% to 13%. Sweetpotato samples (n = 330) were collected from fifteen commercial fields based on viral symptoms. Virus testing results demonstrated that mixed potyvirus infections were predominant and SPLCV was not detected. SPCSV testing is underway. This study showed evidence of the wide occurrence of potyviruses and a begomovirus affecting sweetpotatoes in NC in research stations and commercial fields. Detection methods used in this study demonstrated the applicability of standardized protocols to investigate virus infection in sweetpotato production areas.

Attempts in extracting RNA from eriophyid mites in search for Rose Rosette Virus

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Phytopathology 104(Suppl. 2):S2.2

Rose Rosette Disease, otherwise known as Rose Rosette Virus, is an ever-increasing problem in Texas, particularly in the Dallas-Ft. Worth area. In 2013, the Texas Plant Disease Diagnostic Lab received thirty-one symptomatic samples of different rose varieties that were tested for Rose Rosette Virus. Of those thirty-one samples, ten returned with positives finds. In the literature, *Phyllocoptes fructiphilus* Keifer, an eriophyid mite has been implicated as the vector of Rose Rosette Virus. To our knowledge, no study has shown the virus to be present within the arthropods. A challenge in this effort has been to find an effective technique/method capable of effectively extracting RNA from the eriophyid mites. The mites themselves are small and tedious to work with. Several simple approaches that would be conducive to the workflow of a diagnostic clinic are explored, all with the purpose of retrieving RNA from the eriophyid mites, and in hopes of being able to detect the Rose Rosette Virus. Methods that have been explored include attempting to harvest the mites off a dark surface background and capturing mites directly in a silica-based spin column in preparation of RNA extraction using commercially available RNA extraction kits. Tissue printing, where the eriophyid mites were squashed onto a filter paper, was also explored as a method to extract RNA. It is clear that more methods, that would fit the requirement of a diagnostic clinic, should be explored to find a simple, consistent and efficient approach to extraction RNA from eriophyid mites and subsequent detections of the rose rosette virus.

Fungicide timing for management of sugar beet diseases in Georgia

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Phytopathology 104(Suppl. 2):S2.2

Sugar beet (*Beta vulgaris*) is not currently grown commercially in Georgia, but production as a winter crop for biofuel is being evaluated. Two major diseases observed are *Cercospora* leaf spot and Southern root rot. Three broadcast sprays of pyraclostrobin (0.11 kg/ha) and fentin hydroxide (0.21 kg/ha) with two banded sprays of prothioconazole (0.20 kg/ha) at various timings gave excellent control of leaf spot, and reduced root rot by more than 50% when applied on a 3-week interval. Stem rot was first observed in April, and was severe by early June. Nonsprayed plots yielded 24,000 kg/ha, approximately half the yield of fungicide-treated plots. Five cultivars evaluated varied significantly in susceptibility to root rot and yield potential. Sugar beets are promising as a rotational crop in Georgia, but further work is needed to refine cultivar recommendations and management programs.

Influence of iron on soybean leaf infection by *Cercospora kikuchii* and symptom expression

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Phytopathology 104(Suppl. 2):S2.2

Cercospora leaf blight (CLB) in soybean, caused by *Cercospora kikuchii*, is a devastating disease in Louisiana and many other states in the southern United States. An alternative method for managing CLB through foliar application of

micronutrients became an important research topic since there are no resistant soybean varieties and fungicide resistance to CLB was reported in 2012. The objective of this work was to document the effects of foliar applications of iron on leaf colonization by *C. kikuchii* and two CLB symptoms, namely blight and purple leaves. Field experiments were conducted in which the cultivar Pioneer 95Y61 was treated with two commercial formulations of Fe, Manny Plex Fe and Fe EDTA (Brandt Consolidated Inc., Springfield, IL), and four rates were applied with a boom sprayer at the R3 and R5 growth stages. Leaf tissue analyses for microelements and qPCR of the pathogen were performed to verify Fe uptake and fungal leaf colonization, respectively. Disease severity was assessed quantitatively for leaf blight and purple symptoms. Results showed a lack of correlation between purple leaf and blight symptoms when the concentration of Fe in the leaves was below 250 ppm; however, above this concentration, severity of both symptoms was reduced substantially. The highest rate of Fe applied (8.33 oz/A) completely controlled the disease. The relationships among leaf colonization as assessed by qPCR analyses, Fe concentration in leaves and disease severity will be discussed.

The complete genome sequence of *Canna yellow streak virus* isolated from Oklahoma grown canna

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Phytopathology 104(Suppl. 2):S2.2

Ornamental canna is produced for worldwide distribution by Oklahoma growers. *Canna yellow streak virus* (CaYSV) is one of the five viruses which causes devastating disease in canna plants resulting in huge economic losses to the growers as well as the state economy. The objective of this study was to characterize the disease symptoms in canna varieties that have red foliage and to sequence the complete genome of the virus. Rhizomes of six canna varieties were planted and maintained in the green house at Oklahoma State University. Diagnostic PCR primers reported by Monger et al., 2007 were used to screen green house plants to identify CaYSV infected plants. Plants were screened by RT-PCR diagnostics to identify a few infected plants. CaYSV infected plants were identified and total RNA was extracted. Five primer sets were designed for the amplification of CaYSV genome using reference genome available in GenBank reported from United Kingdom. One-step RT-PCR was conducted and the PCR products were ligated to pCR2.1 plasmids and transformed to chemically competent *E. coli* cells. Plasmid DNA was sequenced. The sequences obtained were analyzed using nucleotide BLAST through SDSC Biology Workbench. Contigs were prepared using CAP3 program followed by genome assembly using DNASTAR software obtained from Lasergene. A full-length complete genome was assembled with a genome organization typical for the genus *Potyvirus*. This is the first complete genome sequence of CaYSV from United States.

Effect of cultivar, fungicide, and delayed harvest on soybean seed quality

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Phytopathology 104(Suppl. 2):S2.2

Achieving high soybean seed quality is an important part of soybean production in Arkansas. Major factors that affect seed quality are cultivar selection, foliar fungicide application, harvest promptness, and the environment. The objective of this study was to determine the effects of cultivar selection, foliar fungicide application, and delayed harvest on soybean seed quality. In 2008, 2009, and 2010, seven cultivars varying in disease susceptibility were treated with a foliar application of azoxystrobin at the R5 growth stage or left untreated. Plots were harvested promptly at maturity and again, three weeks later. Prompt and delayed harvests were only achieved in 2008. Only delayed harvests were assayed in 2009 and 2010. Standard germination (SG), accelerated aging (AA), and Seed Vigor Imaging System (SVIS) assays were conducted on harvested seed. Seed infection was determined by plating on potato dextrose agar. In 2008, seed infection was low and there was generally a reduction in SG and AA with the delay in harvest, but not in SVIS. Infection of some cultivars increased with delay in harvest. In 2009, seed infection was high. SG, AA, and SVIS were higher in 2009 than 2008 with the cultivars UA4805, AG4403, and Osage, but lower with AP350, MO/PSD-0259, PI80837, and Sween97. In 2010, hot, dry weather resulted in low levels of seed infection. Cultivars MO/PSD-0259 and Sween97 had higher SG and AA in 2010 than 2009. SVIS was slightly higher with MO/PSD-0259 in 2010 than in either 2008 or 2009, while lower with other cultivars. Fungicide application did not consistently affect seed quality tests or infection levels. Delayed harvest, increased seed infection levels by pathogens, and environmental stress appear to have a negative effect on seed vigor.

Evaluating the influence of fungicides with different mode of action on *Cercospora sojina* populations

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Frogeye Leaf Spot (FLS) disease in soybeans is caused by the fungal pathogen *Cercospora sojina* Hara. FLS is most severe under warm, humid conditions. The foliar disease is characterized by gray-brown lesions with darker margins, but stems, pods, and seeds can also be affected. Yield loss as high as 60% has been observed. Historically, FLS has been adequately controlled with resistant cultivars and the use of foliar fungicide applications; however, the surge in quinone outside inhibitor (QoI) resistant *C. sojina* indicates that new management strategies need to be identified. Unfortunately, effective management strategies cannot be designed without identifying the factors which contributed to the selection of fungicide-resistant strains of the pathogen. Understanding the selection pressures exerted by fungicides with different modes-of-action on naturally occurring *C. sojina* populations, could contribute to the design of more efficacious and, potentially, longer-lasting management strategies. An FLS susceptible soybean cultivar (AG 4832) was grown on four no-till fields sites in West Tennessee and one in Southern Illinois, all fields except one had a history of QoI resistant *C. sojina*. The fungicides evaluated include quinone outside inhibitors (QoI), demethylation inhibitors (DMI), succinate dehydrogenase inhibitors (SDHI), methyl benzimidazole carbamates (MBC), and a chloronitrile. All treatments were applied at the beginning pod (R₃) stage of development. The treatment effects were evaluated by assessing the treated plots for QoI-sensitivity of *C. sojina* isolates, FLS severity, and soybean yield. The results will be discussed in the conclusion.

Exploring new diseases at the Texas Plant Disease Diagnostic Lab

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Phytopathology 104(Suppl. 2):S2.3

The Texas Plant Disease Diagnostic Lab (TPDDL) receives over 2000 samples for diagnosis annually. On occasions new types of plants will be seen accompanied by disease problems that are new to Texas. In summer of 2013, the TPDDL received sample submission of dragon fruit cactus plants, *Hylocereus costaricensis*, for diagnosis of a disease problem that affected fruit and leaves. Dragon fruit is a highly nutritious fruit from several species of cacti. These plants are being evaluated as a potential cash crop by some growers in South Texas. Symptoms observed on the submitted plants included brownish black spots that eventually coalesced on fruit, forming dark, depressed soft rot regions. Under moist conditions, dark fungal spore mass can be observed growing from these lesions. On the stem or leaf cuttings, small tan scabby lesions with a lateral fissure were observed. These are typically discrete lesions and can also produce dark spore mass when incubated in a moist chamber. The fungus that was isolated from these lesions was putatively identified as *Bipolaris cactivora* based on conidiophore and conidia morphology. Further work is being done to confirm the identification of this fungus. To satisfy Koch postulate, pathogenicity test was done on stem/leaf tissue using a detached leaf assay method. Additionally, young leaves from potted plants were inoculated to test for pathogenicity.

Prevalence and control of pathogenicity groups of *Leptosphaeria* spp. from winter canola in Oklahoma

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Phytopathology 104(Suppl. 2):S2.3

Black leg, caused by the fungus *Leptosphaeria maculans*, is an economically important disease of canola (*Brassica napus*) first detected in Oklahoma in 2009. Strategies for management of the disease are deployment of resistance genes, application of fungicides, and cultural practices. Resistance genes in canola (*Rlm*) interact with avirulence genes in the fungus (*AvrLm*) in a gene-for-gene manner. Little is known about the local distribution of pathogenicity groups (PGs) or avirulence genes. Isolates (109) of *Leptosphaeria* spp. from different fields were assigned to four PGs and probable races based on the phenotype interaction on cotyledons of the differential cultivars Westar, Glacier (*Rlm2*, *Rlm3*) and Quinta (*Rlm1*). PG1, avirulent on Westar and recognized as *L. biglobosa*, was found at a frequency of 47%. PG2, virulent only on Westar, represented 5% of the isolates. PG3, virulent on Westar and Glacier, represented 14% of the isolates. PG4 virulent on all differentials was found at 34%. In order to test canola germplasm for resistance to local strains, the effect of fungicide seed treatment and its removal on disease expression on

cotyledons was determined. Seeds of the cultivar DKW46-15 that were commercially treated with Prosper FX were washed twice in soap and distilled water on a rotary shaker for 30 minutes. Cotyledons from untreated, treated, and washed seeds were inoculated with *L. maculans* isolates. There was no significant difference in disease severity (1-6) between washed (3.4) and untreated (3.4) seeds. Disease severity was significantly lower (2.6) on cotyledons of treated seeds, but the disease was not completely controlled by the seed treatment. Results demonstrated that the virulence genes *avrLm1*, *avrLm2*, and *avrLm3* are common in local strains of *L. maculans*; and that is possible to test for seedling resistance in cultivars with seed treatment.

The initial development of a weather based risk model for early season fungicide sprays of *Sclerotium rolfsii* in peanuts

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Phytopathology 104(Suppl. 2):S2.3

Stem rot or white mold, caused by *Sclerotium rolfsii* Sacc., is a devastating disease of peanuts in the Southeastern U.S. Typically this disease is managed with fungicide specific sprays starting at 60 days after planting (DAP). However, recent fungicide experiments have indicated that *S. rolfsii* can be more efficiently managed with early season sprays applied between 20 and 40 DAP. The objective of this study was to develop a preliminary model that uses readily available soil temperature and rainfall weather data to assess early season peak growth periods of *S. rolfsii*. A soil temperature ratio was developed to compare the number of hours of non-optimal and optimal soil temperatures. The ratio was calculated by dividing the number of hours that soil temperatures were less than 24 C by the number of hours it was between 27–35°C. Ratio values < 0.5 for a 24 hour period were considered optimal for fungal growth. Moisture is also needed for fungal growth and development. Thus, the temperature ratio was combined with rainfall data to determine *S. rolfsii* growing degree day. Adequate moisture was considered to be present if a rainfall event of greater than or equal to 1.27 cm occurred within the last 5 days, or great than or equal to 2.54 cm occurred within the last 10 days. Initial assessments indicate that the model tended to over predict *S. rolfsii* disease development at 3 out of 4 field sites during the 2013 growing season. However, the inoculum pressure in these sites was considered to be low as indicated by the untreated check. Future analysis will be conducted to refine the model parameters and combine the outputs with Peanut Rx.

Interactions of seed-borne bacterial pathogens with host and non-host plants in relation to seed infestation and seedling transmission

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Phytopathology 104(Suppl. 2):S2.3

The bacteria *Acidovorax citrulli*, *Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas euvesicatoria*, and *P. syringae* pv. *glycinea* were cross-inoculated in to watermelon, tomato, pepper, and soybean. Seed-borne infestation and transmission were assessed for both compatible and incompatible combinations. Seed-borne infestation and transmission were also assessed for onion (incompatible host with all bacteria listed above). A non-pathogenic, pigmented strain of *Serratia marcescens* was used as a control. Blossoms were inoculated with 1×10^6 colony forming units (CFUs)/blossom of each bacterium. No symptoms developed from blossom-inoculated plants for either compatible or incompatible combinations. Seeds harvested from each pathogen-host and -non-host combinations were assayed for bacteria by plating on semi-selective media. Bacteria were detected on semi-selective medium for all bacteria-host and -non-host combinations and further confirmation of isolated colonies was done with specific polymerase chain reactions. Additionally, seeds resulting from blossom colonizations were assayed for transmission by a seedling grow-out assay (SGO) in the greenhouse (28°C and ≥80% R.H). The percentage of seed infested was 4–100% and was not significantly different between host and non-host combinations. Furthermore, the transmission of pathogen from host and non-host seeds to seedlings occurred in both a symptomatic and an asymptomatic (epiphytic colonization) manner, respectively. The percentages of pathogen transmission to host and non-host seedlings varied from 3.5 to 64.5%. Seeds from blossoms inoculated with *S. marcescens* were infested (24.5%) and the bacterium was transmitted as an epiphyte (13.2%). These results indicate that the seeds of non-host plants can become infested with incompatible bacterial pathogens through blossom colonization and they can be transmitted via epiphytic colonization of seedlings. Consequently there is not only a risk of interstate and international movement of compatible seed-borne pathogens but there may also be a potential risk of movement of incompatible plant pathogens through seed-borne infestation via blossom colonization.

Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to fluopyram

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Phytopathology 104(Suppl. 2):S2.4

Fluopyram is an SDHI fungicide that is being evaluated as a seed treatment and in-furrow application on row crops for management of fungal diseases and its effect on plant-parasitic nematodes. Currently, there is no LD₅₀ value, data on nematode recovery, or effects of sub-lethal concentrations of fluopyram on nematode infection for *Meloidogyne incognita* or *Rotylenchulus reniformis*. Only 24-hr-old J2 of *M. incognita* collected from a hatching chamber and 48-hr-old mixed-life stages of *R. reniformis* collected by a modified Baermann funnel method were used in these sensitivity assays. Paralysis was observed after 2 hr of exposure at 1.0 µg/ml fluopyram for both nematode species. Using an assay of nematode motility, LD₅₀ values of 5.19 µg/ml and 12.99 µg/ml were calculated based on 2 hr exposure for *M. incognita* and *R. reniformis*, respectively. Recovery in nematode motility was observed for both nematode species from a 1 hr exposure at their respective LD₅₀ concentration with a higher proportion of recovery for root-knot than the reniform nematode. Exposing *M. incognita* for 1 hr at sub-lethal concentrations of 5.2 and 3.9 µg/ml resulted in significant reduction on the infectivity of tomato roots based on a root-gall index. Though further studies are needed, these data suggest a very unique response by root-knot and reniform nematodes to fluopyram.

Xylella fastidiosa infection is correlated with lower yield in a rabbiteye blueberry orchard in Louisiana

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Phytopathology 104(Suppl. 2):S2.4

Research in Georgia and Florida has confirmed *Xylella fastidiosa* as the cause of a leaf scorch disease of southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids), but an inoculation experiment suggested no or limited impact of the bacterium on rabbiteye blueberry (*V. virgatum*) cultivars. However, in Louisiana and Mississippi, *X. fastidiosa* has been detected by ELISA and/or real-time PCR in rabbiteye plants from orchards that have experienced plant decline and/or death. In an effort to determine whether infection was correlated with yield loss, harvest data were collected at an established orchard with *X. fastidiosa*-infected rabbiteye blueberry plants. In 2013, infected rabbiteye cultivar Tifblue plants (n = 9) yielded an average of 4.0 kg of fruit with an average berry weight of 1.2 g, while plants in which the bacterium was not detected (n = 32) yielded an average of 8.6 kg of fruit with an average berry weight of 1.4 g. Significant differences between infected and non-infected plants were detected for both total yield and average berry weight. Research on the effects of *X. fastidiosa* infection on, and prevalence in, rabbiteye blueberry appears warranted. Collection of yield, plant infection, and survival data will continue in 2014.

Alternative hosts of spring dead spot-causing fungi

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Phytopathology 104(Suppl. 2):S2.4

Three fungi in the genus *Ophiosphaerella*, *O. herpotricha*, *O. korrae* and *O. narmari*, are the causal agents of spring dead spot of bermudagrass. These fungi can cause disease in grasses other than bermudagrass, including zoysiagrass and buffalograss. However, the extent to which *Ophiosphaerella* spp. can colonize and infect different hosts is unknown. To determine the potential host range of *Ophiosphaerella* spp. several warm-season and cool-season grasses and a dicot were evaluated for colonization by *O. korrae* and *O. herpotricha*. Plants were grown from seed under greenhouse conditions. Roots were washed with water, inoculated with either *O. herpotricha* or *O. korrae*, and incubated at 17°C. Intact and transverse sectioned roots were examined under a fluorescence microscope at 2 to 14 days after inoculation. The interaction with different plant species was similar for both fungal species. In creeping bentgrass, the fungi was able to colonize the root vasculature without causing necrosis. In zoysiagrass and buffalograss, the fungi occasionally caused necrosis of the root cortex, but did not colonize the vasculature. Proso millet and blue panicgrass exhibited cortical colonization, but no visible necrosis. Broadleaf panicum, big bluestem and alfalfa were non-hosts, with minimum root surface colonization. *Ophiosphaerella* spp. appear to have a broader host range than previously recognized, though many of the plant-fungus interactions do not result in disease-associated necrosis.

Triggering toxin production: The cellobiose signaling pathway leading to virulence of *Streptomyces scabies*

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Phytopathology 104(Suppl. 2):S2.4

Thaxtomin A is the primary virulence determinant of *Streptomyces scabies*, the major scab-causing pathogen on several important root and tuber crops responsible for considerable annual losses worldwide. It is the predominant form of a family of nitrated dipeptidic phytotoxins and acts as a cellulose synthesis inhibitor in expanding plant tissue, therefore enabling the bacterium to infect living plants, a rare trait among the commonly saprophytic streptomycetes. Toxin production is induced by cellobiose, the smallest subunit of cellulose, a major plant cell wall component. We identified the mechanisms by which *S. scabies* can sense the presence of a host and translates this information into the activation of thaxtomin production and therefore virulence. Through mutational and transcriptional analysis, we demonstrated a role for two ABC-transporters and their respective regulators in thaxtomin production. One of these regulators is of particular interest since two specific binding sites are associated with the thaxtomin biosynthetic cluster. Manipulation of this regulator allows for production of thaxtomin A without the need for cellobiose or other cellulose components in the culture medium. Our work shows a remarkable example of how *S. scabies* evolved to recruit a common metabolic pathway to fine-tune its main virulence mechanism for plant root and tuber colonization by inhibiting plant cellulose synthesis.

Nematodes associated with edamame, *Glycine max* L. (Merr.) in Arkansas

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Phytopathology 104(Suppl. 2):S2.4

Edamame, also known as vegetable soybean, *Glycine max*, was introduced to the United States from Japan in 1890 and has been growing in popularity as a high-fiber, low-sugar snack in recent years. In 2012, The American Vegetable Soybean and Edamame, Inc. (AVS) established the first commercial processing plant in the United States near Mulberry, AR. About 2,000 acres of edamame are now being grown annually in the area. Since edamame is harvested as an immature seed, management practices are different from those used for conventional soybean. Plant-parasitic nematodes, particularly *Meloidogyne incognita* (southern root-knot) and *Heterodera glycines* (soybean cyst) known to be wide-spread in Arkansas, are one of the pests of concern. The objective of this study is to determine the nematodes associated with edamame in Arkansas fields. Edamame production fields of 2013 were surveyed immediately following harvest to determine the presence, identity, and relative population density of nematodes. Where they were detected, *Meloidogyne* spp. second stage juveniles were collected and used to establish greenhouse populations on tomato for further identification to species. Additionally, edamame lines developed by the Arkansas soybean breeding program were evaluated for host suitability to *M. incognita* and *H. glycines* in greenhouse trials. All lines were good hosts for *H. glycines*, race 3, but *M. incognita*, race 3, reproduction was significantly lower in one of the studied lines. This work will aid producers and crop advisors in developing nematode management strategies for edamame production and provide insight on the potential risk of root-knot and soybean cyst nematodes to this emerging crop.

Evolutionary relationship among species of the *Pythium irregulare* complex

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Phytopathology 104(Suppl. 2):S2.4

Pythium irregulare is an important plant pathogenic species complex with a broad host range, and is the most prevalent *Pythium* affecting ornamental hosts. Two cryptic species, *P. irregulare sensu stricto* (s.s.) and *P. cryptoirregulare*, have been described within the complex, based on morphology, two DNA sequences, and AFLP fingerprints. However, additional groups have been tentatively identified within *P. irregulare* that require further analyses to resolve their phylogenetic status, including *P. 'vipa'*. To evaluate this issue, an international collection of 92 isolates collected from diverse host, was characterized using a multilocus approach. Phylogenetic analyses were performed on DNA sequences of the internal transcribed spacers region of the ribosomal DNA including the 5.8S unit (ITS), β-tubulin (Btub), heat shock protein 90 (HSP90), and cytochrome oxidase subunit I, including the cox I-II spacer (coxI-II). A subsample of 41 *P. irregulare* s.s. and *P. cryptoirregulare* isolates from NY were examined by microsatellite analysis of 11 polymorphic

loci to assess interspecies boundaries. Multiloci DNA sequence analysis revealed significant genetic differentiation within the species complex. DNA barcodes varied in their power to resolve closely related species within the *P. irregulare* species complex. HSP90 was the least informative barcode, providing no resolution among the species. Btub resolved *P. 'vipa'* with low support, but grouped *P. irregulare* s.s. and *P. cryptoirregulare* together. ITS and coxII resolved *P. irregulare* s.s., *P. cryptoirregulare* and *P. 'vipa'*. Microsatellite analysis supported an interspecific boundary between *P. irregulare* and *P. cryptoirregulare*. The results of this study supported the species complex status of *P. irregulare* and provided additional support for *P. cryptoirregulare* and *P. 'vipa'* as species distinct from *P. irregulare* s.s.

Site specific application of fumigants for control of nematodes in Louisiana and Texas

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Phytopathology 104(Suppl. 2):S2.5

Management recommendations for plant parasitic nematodes are based on species, population density, soil types, nematicide efficacy, economics, and other variables. Research results show that the use of soil maps and apparent Electrical Conductivity (EC_a) data can be used effectively to identify low and high risk areas, or "responsive" zones for root-knot nematodes (*Meloidogyne incognita*). As a result, input costs for the application of Telone II soil fumigant can often be reduced by 30 to 40% by using this technological approach to assist in nematode management. Advances in EC_a data collection, Geographic Information Systems (GIS), Global Positioning Systems (GPS), application equipment, and an increase in user expertise has enabled broader adoption and investigation of cotton yield responses. These studies were conducted to further define the value of site specific applications in fields with variable nematode populations and soil types in Louisiana and Texas.

Goss's wilt in Louisiana: Incidence, severity and loss

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Phytopathology 104(Suppl. 2):S2.5

Goss's wilt, a bacterial disease caused by *Clavibacter michiganensis* subsp. *nebraskensis* was confirmed in Louisiana for the first time during the 2013 corn growing season. Field symptoms were found in mid-June in East Carroll, Madison, Tensas and West Carroll Parishes in the northeastern area of the state. Classical foliar and stalk symptoms were observed with the greatest damage occurring in the center of 50 to 75 ft. diameter circles, while less severe symptoms and, therefore, less damage occurred at circles' edge. The bacterium was isolated from symptomatic tissue on CNS selective agar media and the colony morphology matched as previously described. Furthermore, *C. m.* subsp. *nebraskensis* specific primers PSA-7 and PSA-R were used to amplify and sequence the PCR product. The blast *n* analysis of amplified PCR product (393 bp) resulted in 100% homology with *C. m.* subsp. *nebraskensis* strain KACC20788 (GenBank Accession No. JN613835). Pathogenicity was performed and the inoculated plants produce similar symptoms as originally described and the control plants remained symptomless. In the field all observations of Goss's wilt were found affecting only three corn hybrids from the dozens of susceptible hybrids grown in the area indicating seed as the probable initial inoculum source. Incidence of Goss's wilt in the affected parishes has been estimated at less than one percent while the severity within the affected circles ranged from 45 to 100%. Yield losses in the affected areas ranged from 50 to 100%. Through education efforts growers responded to the new disease by burning and burying crop residues. Their plans for the 2014 growing season include rotating with a non-host (soybean) in affected fields.

Effects of irrigation systems and bed and row middle fumigation on the incidence and severity of diseases caused by *Fusarium oxysporum* in tomato

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Phytopathology 104(Suppl. 2):S2.5

In Florida, several diseases caused by soilborne pathogens have reemerged with the adoption of alternative soil fumigants to methyl bromide (MeBr) such as Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *lycopersici* FOL), and Fusarium crown and root rot (caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). Colonization from non-fumigated row middles and under-fumigated regions within the bed are likely sources of inoculum for the infestation of raised beds. We hypothesize that because the alternatives are less volatile than MeBr, the effective zone of fumigation is reduced promoting

early bed re-colonization. Trials were initiated in February 2013 to define the effective zone of fumigation in a standard 1,3-D + Pic fumigated, raised bed system, and the effect of irrigation and additional row middle fumigation based on the recovery of total *Fusarium* spp. Four sets of soil cores were taken in each plot over the course of the season from 16 different locations throughout the bed and row middles ranging from 2 to 10 inches deep to assess the distribution and quantity of total *Fusarium* spp. using standard dilution plating techniques on a semi-selective medium. Although plots were inoculated with FOL and FORL in the first season, disease incidence was low and no differences were found for the effect of irrigation (drip vs. seepage). Bed location, time, bed fumigation, row middle fumigation and interactions within all had significant effects on total *Fusarium* levels. Fumigated plots had significantly lower levels of total *Fusarium* spp. than non-treated plots, but no differences over time within the fumigated plots. However, there were significant location differences within plots at each sampling period.

Discovery of a white false smut infecting rice in Arkansas

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Phytopathology 104(Suppl. 2):S2.5

False smut of rice (*Oryza sativa*) caused by *Ustilaginoida virens* (teleomorph: *Villosiclava virens*), is increasingly prevalent in rice fields of Arkansas. In Japan and China, green and white false smuts occur on susceptible rice cultivars. White rice false smut was named as a new species, *Ustilaginoida albicans* in 1997. In 2011, 2012, and 2013, smutted rice panicles with white sori were found in rice variety resistance tests at the Newport and Pine Tree Agricultural Experiment Stations in Arkansas. The identity of the white smut we found was studied by morphological and rDNA comparisons between white and green false smut isolates from rice in Arkansas. Conidia and sori morphology, rDNA amplified with universal internal transcribed spacer (ITS) primers ITS1/ITS4, and inoculations to rice cultivars were performed to identify of the causal agent. The morphological examinations indicated that unlike green smut, the conidia from the white false smut lacked cell wall pigmentation and spines on the surface. Pairwise comparisons of the sequences of ITS 1-4 rDNA from isolates of both smuts did not separate the white or green false smut isolates from each other. Virulence tests confirmed that one white smut isolate (I-9E) infected rice and produced sori and conidia on panicles similar to the sori and conidia from the white false smut sori and conidia found in fields at Newport and Pine Tree. Work to identify the isolates obtained from white false smut sori in this study is ongoing. To our knowledge, this is the first report of white false smut occurring in Arkansas.

A novel plasma seed treatment for a rice seedborne fungal pathogen

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Phytopathology 104(Suppl. 2):S2.5

Seeds contaminated with pathogens are the primary inoculum for plant diseases in many food crops. Conventional treatments for seed-borne diseases use hot water, chlorine or fungicide applications. A novel seed treatment method based on non-thermal plasma generated by an air dielectric barrier discharge (DBD) device was evaluated in this study as an alternative to these conventional treatments. Non-thermal plasma at atmospheric pressure and room temperature consisted of partially-ionized gases, which are chemically reactive. The antimicrobial activity of non-thermal plasma was evaluated against an important seedborne pathogen, *Gibberella fujikuroi* that causes bakanae disease in rice. Non-thermal plasma treatments effectively inhibited the growth of *G. fujikuroi* on nutrient growth medium and reduced the number of fungal colony forming units (CFU) on the rice seed surface by >92% at 120 s exposure. Effective exposure times to 50% (ET₅₀) and 90% (ET₉₀) control of *G. fujikuroi* CFU on the seed surface were 9.0 and 76.3 s, respectively. Ten minutes of non-thermal plasma treatment on seeds infested with *G. fujikuroi* significantly reduced disease development. No adverse effects were detected on the seed germination rate and seedling height when uncontaminated seeds were treated with non-thermal plasma for up to 20 min. The treated seeds germinated and grew similarly compared with non-treated uncontaminated seeds. This study indicates that non-thermal plasma possesses antifungal activity and showed potential as an effective disinfection technique to reduce the contamination of seedborne fungal pathogens from seed surface without compromising seed health.

Evaluation of commercial cultivars of soybean in response to *Cercospora sojina* under a regime of foliar applied fungicide

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Phytopathology 104(Suppl. 2):S2.5

Frogeye leaf spot (FLS) is the result of infection from the fungus *Cercospora sojina* Hara. Infection due to this disease can lead to dramatic losses of photosynthetic area on green leaves, premature loss of leaves, and can lead to compromised stems, pods, and seeds. Yield losses ranging from 20–40% are not uncommon in years where weather conditions are favorable for fungal growth and cultivars lack genetic resistance. Producers, in areas where FLS has historically caused yield losses, have attempted to combat the disease with cultivar selection and a foliar fungicide spray regime. To determine how many commonly available cultivars respond to FLS under a foliar fungicide treatment, we selected available cultivars over a 6 year span and applied a foliar fungicide at growth stage R3. We monitored the presence of FLS on sprayed and unsprayed plots by rating plots based on the percent area affected. We also collected yield on all plots to determine what affect fungicide application had on the yield of each variety. Results based on this research continue to support that some of the most useful techniques for producers are to use cultivar selection coupled with an appropriate spray regime.

Detection and quantification of *Cercospora janseana* by Real Time PCR technique

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Phytopathology 104(Suppl. 2):S2.6

Cercospora janseana is the causal agent of an economically important disease of rice 'Narrow Brown Leaf Spot of Rice (NBLS)'. In the decade, this disease has become a serious threat to the rice industry of Louisiana, Texas, Mississippi and Arkansas. In optimum conditions, this disease can cause more than 40% yield loss. Little information about the biology of the pathogen and disease is available in scientific literature. The Crop Loss Assessment Laboratory of Department of Plant Pathology & Crop Physiology in collaboration with Rice Research Station, Crowley have initiated studies to understand the biology of the pathogen and the integrated management of NBLS. A study was conducted to develop a protocol for early detection of the pathogen and quantification of *C. janseana* in varieties with different susceptibility levels, fungicide treated and non-treated plants and at different planting dates using real time PCR. For detection, DNA extraction of pure culture of *C. janseana* was done. Conventional PCR was used with universal primers and gel electrophoresis to find the size of the PCR product. After PCR product purification and sequence analysis, sequences were aligned and blast with NCBI data base. Blast search showed the highest similarity with partial cds region of polyketide synthase gene of *Spherulina oryzae* (teleomorph of *C. janseana*). Specific primers and probe for real time PCR were designed based on that region. To quantify the inoculum build up over time in treated and non-treated varietal plots at 3 different planting times, samples were collected at different time periods over the crop season. Results showed significant variations in level of inoculum among the varieties, planting dates and fungicide treatments.

Meloidogyne partityla, a new root-knot species to Arkansas

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Phytopathology 104(Suppl. 2):S2.6

Pecan (*Carya illinoensis*) is a valuable nut tree belonging to the family Juglandaceae. It has been reported that pecan orchards of Georgia, New Mexico, Florida, Texas, Arizona and Oklahoma are infected by the root-knot nematode, *Meloidogyne partityla*. A survey of several Pecan orchards in Arkansas was made during the month of September 2013. Soil samples from Pecan Orchards in Pope (near Russellville), Logan (near Paris) (near the Arkansas River) and Miller Counties (near the Red River) were taken. To determine the presence of root knot nematodes the soil samples were processed using the sugar floatation method. Root-knot juveniles (J2s) were obtained from the Logan and Miller County sandy soil samples. No females or males were found in the soil or root samples. Single juveniles were hand-picked looking at the stereo microscope and kept in water drop on the glass slide. For molecular studies juveniles were squashed using a micropipette tip and the solution obtained was used to run Polymerase Chain Reaction (PCR). The primer set used was C2F3/1108 which amplifies the region between COII and 16S ribosomal mitochondrial genes. The amplified product was purified using Nanosep 30K column and sent to the UAMS DNA Sequencing Core Facility, Little Rock, Arkansas. Sequences obtained were compared with non-redundant nucleotide sequences in Genbank through NCBI BLAST. The blast result confirmed the root-knot species in Logan County to be *M. partityla*. This is the first report of *M. partityla* in Arkansas. Reports from J2 from Miller County are still pending. Tomato plants were inoculated in greenhouse tests to eliminate the possibility of it being *M. arenaria*, *M. hapla* *M. incognita*, or *M. javanica* and no reproduction of root-knot nematode was found to occur. Morphometric and host range studies on this root-knot species are in process.

Variation in virulence of *Sclerotium rolfsii* isolates from peanut in Florida

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Phytopathology 104(Suppl. 2):S2.6

Stem rot, caused by *Sclerotium rolfsii* Sacc., is a serious disease of peanuts in Florida and throughout the Southeast causing yield losses as high as 80%. Production practices such as variety selection, fungicide usage and crop rotation have created selection pressures for *S. rolfsii* populations within the different production regions of Florida. The objective of this study was to assess the variation among *S. rolfsii* isolates from different regions of Florida in terms of their virulence. Greenhouse and field virulence tests were conducted by inoculating fifteen different *S. rolfsii* isolates onto the peanut cultivars Georgia-06G (susceptible) and York (resistant). A split plot design was used to conduct the experiment with variety as main plot factor and isolate as sub plot factor. Peanut plants were inoculated with a 1 cm diameter actively growing mycelial plug and wrapped around the main stem with Parafilm. A total of 4 and 5 consecutive severity ratings were collected in the greenhouse and field experiments, respectively. There was a significant ($p < 0.05$) variation in virulence among the isolates tested in this study with severity levels differing by as much as 23% between isolates. Furthermore, in the field experiment isolates were observed to be more virulent on Georgia 06-G with average final severity of ~19% compared to ~8% on York. However, the same host response was not observed in the greenhouse experiments with the average final severity being ~36% on Georgia-06G and ~33% on York. Our results indicate that there is a high diversity in virulence of *S. rolfsii* populations within Florida despite regional production selection pressures that could reduce this diversity. Future research is needed to determine the true extent of this diversity using more extensive sampling protocols and molecular approaches.

Survey and detection of *Phytophthora ramorum*, the Sudden Oak Death pathogen, in Texas

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Phytopathology 104(Suppl. 2):S2.6

In 2004 the Sudden Oak Death (SOD) pathogen, *Phytophthora ramorum*, was first detected in Texas on nursery stock in 11 out of 112 surveyed "trace forward" nurseries. Those discoveries stimulated surveys to determine whether the pathogen had subsequently become established in the nurseries or surrounding vicinities. Survey methods consisted of sampling nursery plants and soils, streams in the vicinities of the contaminated nurseries, and nursery perimeters for potential infections of known or related hosts of the pathogen. Depending on the objectives and types of samples, pathogen detection was carried out by a combination of methods, including isolation on PARP media, ELISA testing, or confirmation by QRT-PCR. From 2004 through 2011, no additional positive detections of *P. ramorum* were made. Other *Phytophthora* spp. were commonly detected in the nursery environments. However, in 2012, soil samples collected from a nursery in Seabrook, TX, tested positive for *P. ramorum* by isolation and QRT-PCR. Further, the stream running next to this nursery was found to be positive at two locations by baiting. In early 2013, a stream running next to a different nursery in Webster, TX, also tested positive. These detections raise questions regarding the epidemiology of this pathogen and the potential for establishment at new locations, particularly where environmental conditions are not considered to be conducive.

Effect of soil matric potential on infection of sorghum seedlings by *Peronosclerospora sorghi*, cause of sorghum downy mildew

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Phytopathology 104(Suppl. 2):S2.6

Sorghum downy mildew (SDM), caused by the oomycete *Peronosclerospora sorghi*, is a disease of world-wide importance occurring nearly everywhere sorghum is grown. Although seedling infection occurs from soilborne oospores, soil factors that promote infection are poorly understood. The objective of this study is to determine how three soil matric potentials influence oospore infection of a susceptible hybrid growing in two soil types. Soils used in this study were Belk clay, a silty clay loam, and Houston black clay. Seeds were planted into oospore-infested soils, which were maintained for two weeks at constant matric potentials using a Haines apparatus. The matric potentials were: 0 bar (saturation), -0.2 bar, and -0.33 bar (field capacity). After incubation, the seedlings were transplanted into pots and grown for one week, to allow for full symptom expression for disease incidence evaluation. Data analysis by logistic regression indicated a significant ($P = 0.01$) effect of matric potential on plant infection. The highest levels of infection (26% to 82%) occurred at -0.33 bar in both soil types, while the lowest levels (0% to 37%) were at 0 bar. There was not a significant interaction between soil type and matric potential. Incidence was higher in

Houston black clay. This information may be useful in improving current integrated management recommendations to control SDM.

Crop protection in algal cultures grown for oil production

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Phytopathology 104(Suppl. 2):S2.7

In order for the production of oil from algae to be commercially successful, cultivation must be in large open ponds. Pest management is a major challenge in this type of system. Algae in open ponds are at danger from weeds, disease, and consumers of algae. Sapphire Energy has developed an integrated pest management system borrowing from many of the principles already in use in traditional agriculture. Pests are identified and tracked in the field. Solutions to mitigate their impact on crop productivity are developed through laboratory research before being deployed in the field when predetermined thresholds are reached. Using this system Sapphire Energy has successfully cultivated the same strain of algae for more than a year in open ponds. Critical to the success of such a crop protection program is an understanding of the biology within the pond ecosystem. Here we describe Sapphire's progress in this area with particular focus on two of our major pests; fungal parasites and weed algae.

Species and strain differentiation of *Aspergillus* section *Flavi* through morphological characterization and repetitive-sequence-based PCR DNA fingerprinting

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Phytopathology 104(Suppl. 2):S2.7

Aspergillus flavus and *A. parasiticus* are major concerns in peanut production due to their ability to produce aflatoxins. Aflatoxins have carcinogenic and immunosuppressive properties that have caused high mortality and reduced productivity in livestock as well as hepatocellular carcinoma (liver cancer) in humans. *A. flavus* produces aflatoxins B₁ and B₂, whereas *A. parasiticus* produces B₁, B₂, G₁ and G₂. Seventy-eight *Aspergillus* isolates collected from field and commercial peanuts in Georgia, Texas and Alabama were characterized to distinguish between these two species. Morphological characterization showed observable differences in their colony color and conidial texture but no obvious differences in growth rate, production of sclerotia and color of sclerotia. From the 78 isolates, 39 were chosen for further analysis using repetitive-sequence-based PCR (rep-PCR) DNA fingerprinting through the DiversiLab system. This approach is based on amplification of different sized fragments representing short repetitive DNA sequences that are dispersed throughout an organism's genome. Since unique fingerprint profiles are created for each organism, this approach can be used to discriminate the isolates to the strain level. Majority of the isolates were identified as *A. flavus*, three as *A. parasiticus* and one as *A. oryzae*. Isolates identified as *A. flavus* showed limited genetic diversity, clustering only into two groups. No grouping according to location of collection was observed.

Detection and quantification of aflatoxigenic fungi in drought-stressed peanut seeds through quantitative real-time PCR

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Phytopathology 104(Suppl. 2):S2.7

Aspergillus flavus and *A. parasiticus* are the two major aflatoxin-producing fungal species cotaminating peanuts (*Arachis hypogaea* L.). Fungal invasion and aflatoxin contamination are enhanced by drought conditions, especially when it occurs during the last three to six weeks of the growing season. The objectives of this study are: to determine the lowest amount of aflatoxigenic fungi that can be detected in peanut seeds; and to assess the relationship between aflatoxin contamination and fungal invasion. Peanut samples were collected from field plots inoculated with *A. flavus* and *A. parasiticus* and subjected to drought conditions for 40 days. Aflatoxin contamination was quantified using the Vicam fluorometry method. From these, four samples each with high and low levels of aflatoxin contamination were chosen. Quantitative real time PCR (qRT-PCR) was used to estimate the aflatoxigenic fungi in the seeds by using primers that target the aflS gene, which is involved in the aflatoxin biosynthesis pathway. Sensitivity tests showed that fungal

DNA as low as 10 pg can be detected. Lower cycle threshold (CT) values, indicating higher detection of fungal genes, were generally observed in peanuts that were highly contaminated with aflatoxin as compared to those with low levels of aflatoxin contamination.

A clinical perspective on public plant health trends in Texas

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Phytopathology 104(Suppl. 2):S2.7

The Texas Plant Disease Diagnostic Laboratory (TPDDL) in College Station, TX is the larger of two plant clinics in the Texas A&M University System. Approximately 3500 plant specimens are submitted and examined in the TPDDL annually. An analysis of the sources and composition of processed specimens provides a snapshot of plant health issues affecting the state. The top three plant categories are turf, ornamentals and trees. The proportion of each of these three categories varies little from year to year. An exception was an increase in tree samples between 2010 and 2012, possibly due to the historic drought in 2011. However, during the drought of 2011, the number of soil samples decreased. The largest group of submitters is from the landscape, ornamental and nursery industries. As with commodities, submitter categories remained relatively consistent each year. Marked increases were seen during 2010 to 2012 in submissions coming from government agencies and Texas A&M AgriLife extension agents, volunteers, and researchers. The numbers, types and sources of submitted samples are influenced by a number of factors. For example, the Texas High Plains Plant Diagnostic Lab serves the panhandle agricultural region and processes a far greater proportion of row crops. In contrast, the TPDDL is increasingly serving an urban clientele in the major metropolitan areas of Austin, Houston and Dallas. A large number of samples were diagnosed with *Phytophthora* spp. due to overwatering practices in urban environments. Take-all-root rot and large patch in residential lawns were also common problems from overwatering. Even with these trends, Texas is a large state covering 8 USDA hardiness zones and the TPDDL saw a vast array of diverse plant species and pathogens. Another progressive influence was surveys for invasive plant pathogens, including those causing citrus greening, plum pox, lethal palm decline, and rose rosette.

Evaluation of methods for controlling nematodes in tomato-cucumber double cropping systems

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Phytopathology 104(Suppl. 2):S2.7

Field trials were conducted in the spring and fall of 2013 in Tifton, Ga. and Citra, Fl. to evaluate control methods for nematodes in tomato-cucumber double cropping systems. The purpose of these field trials was to determine the effect of first crop nematode control strategies on nematode populations and damage on a second crop. Tomatoes were planted on April 8 (Tifton) and April 9 (Citra) on raised, black-plastic mulched beds arranged in a randomized complete block design with 6 replications. Treatments for the spring trials included: 1,3 dichloropropene (12 gal/ac), fluensulfone (85.5 oz/ac), resistant cultivar ('PS 01522935'), and an untreated control. 1,3 dichloropropene was soil-injected using a Yetter injection rig and fluensulfone was pre-plant incorporated (PPI). 'Tribute' was used as the nematode-susceptible cultivar. Gall ratings were recorded on June 24 (Citra) and July 8 (Tifton). 1,3 dichloropropene, fluensulfone and the resistant cultivar decreased root galling by 91%, 63%, and 99%, respectively in Tifton and by 84%, 88%, and 100% in Citra compared to the untreated control. Spring tomato plots were split with the tomato spring treatment being the main plot and the cucumber treatments being subplots. Cucumber plots either received fluensulfone (85.5 oz/ac) via drip injection or were left untreated. The cucumber, variety 'Impact', was planted on August 14 (Citra) and August 15 (Tifton). In Citra, plots that had resistant tomatoes and plots receiving MCW PPI in the spring followed by MCW drip in the fall had significantly less galling than the untreated check. In Tifton, plots receiving 1,3 dichloropropene and the resistant cultivar in the spring, regardless of fall treatment, had significantly less galling than the untreated check. Results from these field trials indicate that nematode control options that are utilized on the first crop play an important role in decreasing nematode damage throughout the season.

Challenges of standardizing molecular detection/diagnostic tests for plant disease diagnostic laboratories

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Phytopathology 104(Suppl. 2):S2.7

Early and accurate detection of pathogens encourages effective management decisions and actions, especially in an era where agricultural commerce and climate change converge to distribute and accommodate new hosts and their

accompanying pathogens. Two National Plant Diagnostic Network (NPDN) surveys (2009 and 2013, unpublished) highlighted requests from NPDN diagnosticians for streamlined PCR protocols for pathogen detection. A collaboration among several NPDN labs and researchers have been focused on developing Standard Operating Procedures (SOP) for high-impact plant pathogens. Challenges for the NPDN labs begin with the diagnostic capability of each lab. Those labs that are capable of performing molecular diagnostics are limited by availability of equipment and/or proper controls for the analysis. This project took a two-pronged approach: (1) identifying proper samples for effective detection and (2) development and adaptation of a simple and standard methods that NPDN diagnosticians could use. Based on the selected model pathogens, sampled plant parts for molecular detection is not a one-size-fit-all. Additionally, each NPDN lab may have their own procedures for plant tissue maceration and PCR, equipment differences due to individual preferences and economic limitations. We endeavored to find methods that may transcend these differences and provide a robust and accurate detection procedure for pathogens of concern.

Genetic analyses of single rust spores facilitated by whole genome amplification

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Phytopathology 104(Suppl. 2):S2.8

Switchgrass rust caused by *Puccinia emaculata* can significantly reduce biomass yield and feedstock quality. This pathogen is an obligate biotroph. So, pure, single-spore cultures cannot be isolated on nutrient media, making molecular characterization of individual genotypes within heterogeneous populations difficult. This study assessed whole genome amplification (WGA) of DNA from single spores to facilitate population genetics studies. An Oklahoma population of *P. emaculata* was maintained on several varieties of switchgrass in growth chamber conditions and urediniospores from this population were used for all experiments. DNA was prepared from spores using two different methods. Either DNA was isolated directly from bulked, whole-population urediniospores, or DNA from single, germinated urediniospores was amplified using a commercial WGA kit. From each DNA prep, three DNA barcode genes (ITS, β Tub and TEF1a) were amplified and cloned. Five clones of each PCR product were sequenced and sequences of each barcode were aligned and compared. All DNA preps produced amplifiable templates and band intensities of PCR products amplified from multispore DNA or single spore WGAs were similar. Importantly, cloned gene sequences from the two types of preps were almost identical, confirming the accuracy of WGA. Cloned barcode sequences within single spore WGAs were almost identical, but with some single nucleotide polymorphisms, indicative of spores' heterokaryotic state. Three previously published simple sequence repeats (SSR) and one original SSR also were evaluated. Genetic diversity was observed across the five single spores. In two SSR loci, two alleles were observed, again indicating some urediniospores were likely heterokaryotic. Based on these results, WGA permitted genetic characterization of single spores within a population and will be useful in future studies characterizing the population biology of *P. emaculata* over its native range.

Screening and characterization of hormetic response of *Sclerotinia homeocarpa* to thiophanate-methyl

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Phytopathology 104(Suppl. 2):S2.8

Hormetic dose-responses induced by stressors are characterized by low-dose stimulation and high-dose inhibition. Although the mechanisms that underlie hormesis remain unclear, validation of this phenomenon may have significant implications for management of plant pathogens. Previous studies have demonstrated that radial growth and pathogenicity of *Pythium aphanidermatum* were stimulated by low doses of fungicides. Intensive use of fungicides for management of plant diseases can result in exposure of resistant isolates to sublethal doses. In this study, the dose-responses of a population of 27 isolates of *Sclerotinia homeocarpa*, the causal agent of dollar spot, were evaluated to detect and estimate the hormetic effect of the fungicide thiophanate-methyl on radial growth. Ten treatments were evaluated, including 9 fungicide concentrations and a fungicide-free control, with three repetitions per treatment. Radial growth was measured and compared among treatments using a Brain-Cousens non-linear regression model, and dose-response parameters were determined. Sixteen isolates displayed hormetic responses with up to 12% stimulation. Future research will assess the effect of fungicide hormesis on total mycelium dry weight and on pathogenicity, and stimulatory responses will be characterized by differential gene expression.

Effects of temperature and time of infection on wheat streak severity

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Phytopathology 104(Suppl. 2):S2.8

Wheat streak mosaic virus, transmitted by the wheat curl mite, is one of the most important wheat pathogens throughout the Great Plains region of the United States and causes severe losses to both yield production and water use. Early planting for dual purpose increases the likelihood of infestation by the mite and increased disease severity due to early fall infection. Studies have shown that delayed planting during cooler temperatures reduces disease severity, possibly due to reduced vector infestation during the fall. However, little is known about the impact of time of infection on yield and water use during the spring season. Therefore, a study was conducted to evaluate the effects of late winter and early, middle, and late spring infection by *Wheat streak mosaic virus* at three different irrigation levels. A significant increase in disease severity and decreases in grain yield and soil water uptake were found in fall inoculated plots. Reductions in disease severity were correlated with increases in grain yield during later spring infections, however reductions in disease severity did not uniformly decrease with subsequent inoculations and were influenced by temperature fluctuations brought on by severe cold spells during the early spring. These findings suggest that reduced infection by wheat streak during late planting may not only be due to delayed exposure to infective wheat curl mites, but that temperature may have an effect on virus infection and disease severity. This information could be useful in modeling for disease loss estimations and design of precision management strategies for control of virus disease during environmental conditions conducive to infection.

Response of terpene biosynthetic enzyme transcript accumulation of selected *Medicago truncatula* accessions to infestation with pea aphid, root-lesion nematodes, or *Phoma medicaginis*

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Phytopathology 104(Suppl. 2):S2.8

Plants produce a wide array of secondary metabolites in response to environmental stimuli like insects and pathogens. Among these compounds are the triterpene saponins. Increased saponin levels in plants are thought to have a protective effect against harmful pests. The objective of this study was to evaluate four accessions of *M. truncatula* with varying profiles and concentrations of saponins in roots and aerial tissues for their responses to biotic stresses. Pest performance and accumulation of transcripts were measured for genes encoding enzymes of terpene biosynthesis following infestation with pea aphids, root-lesion nematodes, or *P. medicaginis*. Accession GRC43 has a high level of saponins in its aerial tissue, and had the smallest population of aphids of all the accessions. Accessions with the lowest levels of leaf saponins had greater susceptibility to infection by *P. medicaginis* than accessions with high levels of leaf saponins. GRC43 had low root saponins and the highest nematode soil population. The other accessions were all statistically similar and had high to intermediate saponin levels and lower nematode populations than GRC43. This suggests GRC43 possibly has additional unknown influences other than saponins on nematode populations. An important enzyme in the saponin biosynthetic pathway is β -amyrin synthase. When challenged with pea aphid, GRC43 showed an increase in accumulation of this transcript, while the other accessions decreased. The various accessions showed differences in transcript levels for many of the enzymes involved in the production of saponins. For example, levels of β -amyrin synthase accessions with high pest infestation and infection generally had a lower transcript accumulation, and moderately infested and infected accessions had a greater accumulation of transcript.

Using three plastic mulch colors to investigate the influence of soil temperature and moisture on southern blight epidemics of plasticulture tomatoes

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Phytopathology 104(Suppl. 2):S2.8

Epidemics of southern blight, caused by the soilborne fungus *Sclerotium rolfsii*, vary greatly with environmental conditions from year to year. However, the relationship between these environmental conditions and disease is not well understood. Field trials were conducted across three growing seasons to better understand the role of soil temperature and soil moisture in southern blight epidemics of plasticulture tomatoes grown in three plastic mulch colors (black, white, and reflective). Disease incidence was recorded weekly, and logit-transformed increases in disease incidence between weekly assessments were used for correlations with averages of selected environmental variables in the preceding time between assessment

periods. Soil temperature was significantly ($P < 0.05$) correlated with disease incidence. Differences in disease progress were observed between plastic mulch colors, whereby warmer growing seasons resulted in more severe southern blight epidemics in white and reflective mulches. Cooler growing seasons resulted in more severe southern blight epidemics in black-colored mulch. Stepwise regression models were constructed using the collected environmental variables and demonstrated a quantitative relationship between soil temperature and disease incidence. These and other variables may be useful to construct a disease forecasting model or advisory in future studies to help growers more effectively manage this disease.

Effect of crop rotation and of soil and plant tissue micronutrients on sour skin incidence in *Vidalia* onion

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Phytopathology 104(Suppl. 2):S2.9

Vidalia onions, *Allium cepa*, are a high-value crop grown in Georgia that experience moderate-to-severe, annual losses to sour skin, a disease caused by the bacterium *Burkholderia cepacia*. Assessments of rotation and double-cropping regimes to reduce populations of *B. cepacia*, a soil borne pathogen, were conducted from 2010 to 2013. Onions double-cropped behind pearl millet (*Pennisetum glaucum*) had more marketable onions than onions following corn, peanut and soybean in 3 out of 4 years. Enumeration of *B. cepacia* populations on a modified, semi-selective medium verified that bacterial populations in pearl millet soil in a closed system were significantly less than populations in other soil-plant combinations. The different crops used in the double-cropping and rotation regimes also affected micronutrient levels in both onion tissues and soils, which apparently impacted sour skin levels. Micronutrient levels in both onion bulbs and soils were significantly correlated with sour skin incidence (onion tissue bulb model: $P < 0.001$ and $R^2 = 0.69$; soil analysis model: $P < 0.001$ and $R^2 = 0.57$). Both tissue and soil regression models contained common elements such as Cu, Fe, Zn and Mn. Salicylic acid concentrations in bulbs improved the R^2 in the tissue model and were significantly correlated with the soil model for soils with no rotation. In both models, as Cu levels increased, sour skin incidence decreased. Furthermore, the Cu:Fe ratio had a partial $R^2 = 0.25$ and Cu had a partial $R^2 = 0.17$ and were the largest single contributors to the tissue and soil models, respectively. To determine the applicability of manipulating micronutrient levels in plant tissues, onions were treated in microplots with different micronutrient combinations throughout the growing season. The best treatment raised the Cu:Fe ratio in onion bulb tissues. Future research on the management of sour skin with prescribed fertility applications and the identification of high risk soils based on micronutrient levels is planned.

Citrus canker: Another hurricane for Louisiana's citrus industry

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Phytopathology 104(Suppl. 2):S2.9

Citrus canker caused by *Xanthomonas citri* subsp. *citri* is a devastating disease of citrus. The disease has caused severe economic losses in all citrus growing regions of the world. Citrus canker was first detected in Florida in 1910. By 1914, it had spread to seven Gulf and Atlantic coastal states including Louisiana. Until recently, the disease had not been seen in Louisiana since 1940. However, it was re-confirmed in the state on 28 June 2013. Citrus is the most popular fruit grown in southeastern Louisiana, and includes a number of varieties such as grapefruits, kumquats, lemons, limes, oranges and satsumas. Commercial citrus production is primarily centered in Lafourche, Plaquemines, St. Bernard and Terrebonne Parishes. After hurricane Katrina in 2005, Louisiana lost a majority of its citrus industry, and the total acreage was reduced from 1330 acres in 2004 to 748 acres in 2006. In 2008 and 2010, citrus greening and sweet orange scab were confirmed in the state, respectively, but these did not pose a major threat to citrus production. Citrus canker has been positively identified in Plaquemines Parish where the majority of commercial citrus industry is located along with Jefferson, Orleans and St. Charles Parishes. A total of 432 trees have been confirmed positive and all of Orleans and portions of Jefferson, Plaquemines and St. Charles Parishes are currently under quarantine. Consequently, the movement of citrus plants, plant parts, clippings or fruits is restricted. At present, the total value of citrus fruit and nursery stock industry in the state is valued around \$10 million. Without effective management options, citrus canker has the potential to effect the survival of Louisiana's valuable citrus industry.

Frogeye leaf spot and potential resistance to azoxystrobin in Mississippi soybean production fields

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Phytopathology 104(Suppl. 2):S2.9

Frogeye leaf spot (FLS) is a disease of soybean caused by the fungus *Cercospora sojina*. This pathogen causes circular to angular spots to develop primarily on foliage. Mature lesions may expand and coalesce to form larger, irregular spots. When lesions cover 30% or more of the leaf surface, blighting occurs, leading to potential yield loss. Quinone outside inhibitor (QoI) fungicides are often used to control frogeye leaf spot, but resistance to this class of fungicides has been confirmed in *C. sojina* isolates from Mississippi. The objective of this study is to survey Mississippi soybean fields, isolate *C. sojina*, and test isolates for resistance to the QoI fungicide azoxystrobin. A survey of frogeye leaf spot in Mississippi was taken during the 2013 growing season with samples collected from 49 soybean producing counties. A sample was taken from individual fields and mono-conidial isolations were made, totaling 103 different *C. sojina* isolates. These samples and a baseline isolate were transferred to a soybean stem-lima bean agar (SSLBA) and placed in a growth chamber to promote sporulation. Conidia were collected in sterile water and the conidia suspension was adjusted to 1×10^5 using a hemocytometer. The conidia-suspension was distributed onto the surface of potato dextrose agar (PDA) plates amended with six different rates of azoxystrobin, plus Salicylhydroxamic acid (SHAM) to prevent an alternative oxidative pathway. The plates were then incubated in the dark for 18 hours at 25°C. Conidial germination was determined for 50 conidia, a conidium was considered germinated if the germ tube was at least as long as the conidium itself. To date resistance has been observed in *C. sojina* isolates from Mississippi soybean production fields.

Effects of soil salinity on growth, reproduction, and virulence of *Pythium* spp. on soybean

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Phytopathology 104(Suppl. 2):S2.9

Increasing salinity is an important factor limiting agricultural productivity worldwide. In addition to direct effects on growth and yield, disease severity also may be affected. This study characterized the effects of soil salinity on seedling disease of soybean caused by *Pythium* spp. Controlled environment experiments used two soybean cultivars which differed in chloride tolerance and soil treated with a CaCl_2 solution to create a range of electrical conductivity (EC) levels. Soil was either not infested or infested with *Pythium sylvaticum* or *P. aphanidermatum* (pathogenic to soybean), or *P. oligandrum* (not pathogenic to soybean). Twenty-one days after planting, seedling stand, growth, and development were assessed. Salinity reduced seedling stand at or above 2.0 dS/m. Leaf number, shoot weight and root altitude decreased at or above 2.0 dS/m. Root volume and root tips decreased at 2.6 dS/m but not at lower EC levels. Stand and shoot weight decreased with *P. aphanidermatum* and *P. sylvaticum* at moderate salinity compared to the control. Root development was stimulated by pathogen infestation at the base EC levels. Salinity had no effect when the nonpathogenic species was used. Cultivars responded similarly across treatments. *In vitro*, salinity decreased zoospore production and motility an EC as low as 1.3 dS/m, while zoospore discharge was reduced at 3.3 dS/m, and zoospore taxis was not significantly affected. Mycelial growth and oospore germination were not significantly affected by EC levels. This research suggests that the damage from pathogenic *Pythium* spp. was greatest at moderate soil salinity levels, but that this was not the result of factors that favored the pathogen.

Characterization of virulence phenotypes of isolates of *Colletotrichum sublineolum* from Arkansas on selected grain sorghum hybrids

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Phytopathology 104(Suppl. 2):S2.9

Sorghum anthracnose, caused by *Colletotrichum sublineolum*, continues to be a serious disease of grain sorghum throughout Arkansas. Previously, several new pathotypes of this pathogen were found within the state. Although characterized to pathotype by inoculation of differential breeding lines, the virulence phenotypes of these new pathotypes to the commercial hybrids being grown in the state were not described. In fully replicated experiments, conducted in a greenhouse, 21 day old seedlings of selected grain sorghum hybrids were inoculated with 10 day old spores obtained from cultures of five selected pathotypes. The seedlings were incubated in dew chambers at 25°C for 24 hours before returning them to a greenhouse for 10 to 14 days. Virulence phenotypes were rated on a scale of 1 (resistant) to 5 (susceptible)

for each of the isolates on each of the hybrids. Results of the experiments show that many of the commercial hybrids were susceptible to isolates representing pathotypes 7, 9 and 12. Most of the hybrids tested were resistant or moderately resistant to an isolate of pathotype 4, a dominant pathotype in the USA. These data may help to explain the differences in the resistance ratings for different hybrids at the different locations within Arkansas and provide information toward developing a comprehensive approach to managing this disease.

Screening demethylation inhibitor (DMI) fungicides for assessing efficacy against citrus black spot (CBS)

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Phytopathology 104(Suppl. 2):S2.10

Copper and strobilurin fungicides are the only recommended fungicides for CBS management in Florida. As a potential alternative rotational mode of action, different DMI fungicides were tested for efficacy against *Guignardia citricarpa*, causal agent of CBS. The objectives were to determine the fungal population response *in vitro* and the baseline sensitivity to potential registered DMIs. Tetraconazole, propiconazole, and difenoconazole have been evaluated in a mycelial growth inhibition assay, using 10 isolates. The concentration range for each fungicide was mixed into 1/2-PDA. The ranges were 0.0005, 0.001, 0.003, 0.005, 0.007 and 0.01 mg/ml for difenoconazole; 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 and 1 mg/ml for propiconazole; and 0.005, 0.01, 0.04, 0.07, 0.1, 1, and 10 mg/ml for tetraconazole. The plates were inoculated with mycelial plugs, incubated at 24°C, under 12 h light/dark, and replicated three times per concentration. After 2 weeks, the plates were scanned and mycelial area was measured with Image-J. The EC₅₀ was calculated, using regression with a 3-parameter sigmoidal curve. The EC₅₀ of 10 isolates for difenoconazole, propiconazole and tetraconazole ranged from 0.00125 to 0.0019, 0.007 to 0.01, and 0.028 to 0.056 mg/ml, respectively. Mycelial growth was observed to be greater under 24 h light compared to complete dark. In preliminary data, the EC₅₀ declined from 0.0018 mg/ml in 24 h light to 0.00052 mg/ml in 24 h dark for difenoconazole. Similarly, for tetraconazole, the EC₅₀ decreased from 0.15 to 0.026 mg/ml. An experiment to study the effect of light on the mycelial growth is in progress. Results obtained from this study will be important to understand the *G. citricarpa* sensitivity to DMI fungicides.

Genomic analysis of *Xanthomonas perforans* strains from tomato production areas in Florida reveals two distinct groups

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Phytopathology 104(Suppl. 2):S2.10

A recent survey on bacterial leaf spot (BLS) of tomato strains in Florida revealed that tomato race 4 (T4) *Xanthomonas perforans* has replaced tomato race 3 in Florida. From this collection of T4 strains, 16 representative strains were subjected to whole genome sequencing using MiSeq Illumina system. Comparison of six conserved housekeeping genes among strains revealed two distinct groups. The first group included 11 strains with identical housekeeping gene sequences to *X. perforans* type strain 91-118, while the second group of 5 strains had *gapA* and *gyrB* gene sequences identical to *X. euvesicatoria* type strain 85-10. The difference between these two groups further extended to the type III effector profiles. In addition to the 11 core type III effectors conserved among all BLS causing *Xanthomonas* species, the second group of *X. perforans* strains had a predicted XopQ effector identical to *X. euvesicatoria* 85-10. Likewise, a transposon insertion was detected in XopAF in the second group of strains, whereas the first group of strains had a frameshift in the predicted protein sequence. The discovery of identical sequences between *X. euvesicatoria* and this second group of *X. perforans* strains suggests that genetic exchange has occurred between the two species.

Early season use patterns of prothioconazole for peanut disease management

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Phytopathology 104(Suppl. 2):S2.10

Early season sprays of prothioconazole have reduced peanut (*Arachis hypogaea*) stem rot (*Sclerotium rolfsii*) at harvest. Their benefit when midseason fungicides are used was evaluated in trials in Tifton, GA at Blackshank (BS) in 2012 and 2013 and the Lang Farm (LF) in 2013. Plots were either not treated or treated with early prothioconazole (0.16 kg a.i./ha) in-furrow (IF), or at 30 days after planting (DAP) in a 5-10 cm band. The

other plots received flutolanil (0.76 kg a.i./ha) alone at 63 and 91 DAP, or in combination with IF or 30 DAP banded prothioconazole. All plots received 5 chlorothalonil sprays for leaf spot control. Final stem rot incidence in untreated plots was 40 and 20% at BS and LF, respectively. Disease incidence in plots treated with prothioconazole either IF or 30 DAP were almost identical, and were 26 and 38% less than the controls at BS and LF, respectively. Pod yields for untreated plots were 3648 and 2945 kg/ha, and averaged 582 and 331 kg/ha higher in prothioconazole plots at BS and LF, respectively. Flutolanil reduced stem rot at both locations, but only the prothioconazole at 30 DAP gave additional stem rot control when applied with the flutolanil. The 30 DAP prothioconazole + flutolanil increased pod yields versus plots treated with flutolanil only at both locations, whereas the IF prothioconazole + flutolanil had similar yields as the flutolanil only plots at both locations. Leaf spot incidence at midseason was reduced by all prothioconazole sprays in all trials, but the differences at harvest were only significant at Blackshank. The 30 DAP banded prothioconazole spray can provide additional stem rot control and yield increases beyond those from a traditional midseason fungicide program.

Effect of crop rotation, location and isolation temperature on *Pythium* spp. population composition in Arkansas

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Phytopathology 104(Suppl. 2):S2.10

Pythium spp. are an important group of pathogens associated with stand losses in soybean and rice. A soybean-rice rotation is common in Arkansas. The objectives of this study were i) determine the effect of continuous soybean and soybean-rice rotation on *Pythium* spp. diversity in three locations in Arkansas and, ii) determine the influence of baiting temperature conditions on recovery of *Pythium* spp. Soils from a soybean-rice and a soybean-soybean rotation were collected from three locations in 2012, placed in cups, wetted to saturation, planted with ten seeds of the soybean cultivar Hutcheson, and incubated at 20°C or 30°C. After three days, seeds were recovered and washed in running water and placed on CMA-PARP+B medium. Hyphal tips were transferred to fresh medium. DNA was extracted, the ITS region sequenced, and a Blast analysis to a curated reference database were performed. A total of 275 isolates were identified representing 25 species. The most frequently recovered species were *P. irregulare*, *P. pereocandrum*, *P. sylvaticum*, *P. corulatum* and *P. spinosum*. In both continuous soybean production and rice-soybean production *P. irregulare* was the most prevalent species isolated. In Pine Tree and Stuttgart, *P. irregulare* and *P. paroeocandrum* were the most isolated species. In Keiser *P. coloratum* and *P. sylvaticum* were the most isolated species. At 30°C, *P. torulosum*, *P. aff. dissotocum*, and *P. oopapilum* were isolated but not at 20°C. *P. irregulare*, *P. pereocandrum*, *P. sylvaticum* were isolated at almost the same frequency from both temperatures. Overall, populations of *Pythium* spp. varied the most among locations, but were influenced by the previous crop and the isolation temperature.

Copper in a copper tolerant environment: Questioning the value of copper for managing bacterial leaf spot and speck in Florida tomato production

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Phytopathology 104(Suppl. 2):S2.10

A survey of 175 pathogenic strains of *Xanthomonas perforans* from tomato field and transplant production sites throughout Florida in 2011 to 2012 revealed the prevalence of copper-tolerance among strains, an increasing frequency of streptomycin resistance, and an overall sensitivity to Kasumin 2L (a.i. kasugamycin). Subsequent field trials in 2012 and 2013 were established to test the grower standard program of copper sulfate + mancozeb (Cu-mancozeb) versus non-copper alternatives of Actigard (a.i. acibenzolar-S-methyl), Firewall (a.i. streptomycin), Kasumin 2L, and Quintec (a.i. quinoxifen) alone or in programs using two or more products for managing bacterial leaf spot (BLS) and speck of tomato. For fall 2012, BLS was initiated with a single copper-tolerant, streptomycin-sensitive strain of *X. perforans*. Weekly applications of the Cu-mancozeb alone or in two-way programs of Cu-mancozeb with one of the non-copper alternatives resulted in significantly higher AUDPC values, higher fruit incidence of BLS, and lower yields when compared to programs based on Actigard alone or Actigard in two-way programs with non-copper alternatives. For spring 2013, BLS was initiated with 6 copper-tolerant *X. perforans* strains, two which were streptomycin resistant, resulting in the failure of programs using streptomycin alone. However, similar to fall 2012, Cu-mancozeb applied alone or in two-

way programs with non-copper alternatives resulted in significantly higher AUDPC values and higher fruit incidence of BLS when compared to programs based on Actigard alone or in two-way programs with non-copper alternatives; although none of the programs had a significant effect on yield. Results not only demonstrate the ineffectiveness of copper programs to manage BLS initiated with copper-tolerant *X. perforans* strains, but also the ability of copper to compromise the efficacy of non-copper alternatives when included in a program.

An evolutionary assessment of ectotrophic root-infecting fungi isolated from ultradwarf bermudagrass roots

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Phytopathology 104(Suppl. 2):S2.11

A decline is often observed on ultradwarf bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) putting greens throughout the late summer and early fall months in the southeastern United States. Root systems appear blackened, rotted, and diminutive in size and are frequently colonized with dark, runner hyphae that are indicative of ectotrophic root-infecting (ERI) fungi. To date, bermudagrass decline (*Gaeumannomyces graminis* var. *graminis*) and spring dead spot (*Ophiophora* spp.) are the only known diseases of ultradwarf bermudagrasses caused by ERI fungi. However, lobed hyphopodia, characteristic signs of *Gaeumannomyces graminis* var. *graminis*, are not continually observed in association with affected ultradwarf samples, signifying that other ERI fungi are accountable for compromised ultradwarf bermudagrass roots. Due to laborious and inconclusive identification efforts associated with traditional diagnostic techniques, molecular-based strategies were employed for this study. A six-gene multilocus sequence analysis (MLSA) was proposed to determine relatedness of MSU-ERI fungal isolates to other taxa within Magnaporthaceae and Phaeosphaeriaceae families (Ascomycota). To date, the internal transcribed spacer (ITS) region has been sequenced and utilized in evolutionary analyses. Phylograms were erected using combined consensus from maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) analyses. These assessments indicate that MSU-ERI isolates share common ancestry with the known ultradwarf bermudagrass pathogens *Gaeumannomyces graminis* var. *graminis* and *Ophiophora* spp. In addition, evolutionary analyses define relatedness between MSU-ERI isolates and *Magnaporthiopsis* spp., indicating the discovery of novel fungi associated with decline of ultradwarf bermudagrasses.

Effects of water stress on bacterial panicle blight of rice

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Phytopathology 104(Suppl. 2):S2.11

A study was initiated in 2012 to test the effects of water stress on rice bacterial panicle blight (BPB) disease. Four bays were planted with a BPB-susceptible variety, Bengal at the recommended seeding rate. Two bays were planted with artificially inoculated seeds while plots in the other two bays were planted with non-inoculated seeds. Water treatments were permanent or intermittent flood. Plots of the intermittent flood treatment were allowed to dry down to a soil moisture content of approximately 40 percent before being re-flooded. Soil moisture was recorded at depths of 2 and 4 inch. In 2013, the intermittent flooding was modified to intermittent flushing and a moderately BPB resistant variety, Jupiter, was added to the test. With intermittent flushing, bays were dried down to about 60 percent for a total of six times. For both years of the study, disease levels were twice as high in flooded compared to water stressed plots. In 2013, there was significant difference in disease incidence between varieties. Yield was lower in the water stressed plots compared to flooded plots which appeared to be mainly from water shortage. Milling quality of Jupiter was more significantly reduced with the water stressed condition than Bengal. Since the disease was sporadic and weather dependent, BPB pressure was low in 2012 and 2013 compared to historical BPB epidemic years of 2010 and 2011. The experiment will be repeated in 2014 as in 2014 to confirm the effect of water treatments on BPB levels using these two rice varieties.

Effects of soil, temperature and seed vigor on soybean seedling diseases

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Phytopathology 104(Suppl. 2):S2.11

Seedling diseases are one of the most important problems in soybean production worldwide. Caused by a complex of pathogens, primarily *Phytophthora sojae*, *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani*, seedling diseases are highly dependent on environmental conditions and may

be influenced by soil properties and seed vigor. To determine the effects of soil, temperature, and seed vigor on soybean seedling diseases and the associated seedling pathogens, high and low vigor 'Hutcheson' soybean seed was planted in raw soil from two Arkansas locations: Fayetteville and Pine Tree. Half of the seed was untreated and the other half was treated with mefenoxam and fludioxonil. The experiment was conducted at 25°C and 32°C. After 14 days, plant stands, shoot and root dry weights, and root discoloration ratings were recorded. The experiment was repeated. While there were differences between runs of the experiment, in general, seed treatment increased stands and root weights and reduced root discoloration. Stands were lower at 32°C than 25°C especially in Fayetteville soil. The number of *Pythium* and *Fusarium* isolates depended on the temperature, soil and isolation from seed or seedlings. From seed, *Pythium* spp. were isolated more frequently than *Fusarium* spp. from Pine Tree soil at 25°C, but at similar frequencies at 32°C. Isolations of both pathogen groups were similar in Fayetteville soil at both temperatures. From seedlings, *Fusarium* spp. were the most frequently isolated pathogen group at 25°C but isolations were less at 32°C in both soils. *Macrophomina phaseolina* was more frequently isolated at 32°C than 25°C and only from seedlings.

Effect of temperature on *in vitro* growth of genetically distinct isolates of *Fusarium oxysporum* f. sp. *vasinfectum*

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Phytopathology 104(Suppl. 2):S2.11

Several races of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) are known to affect cotton (*Gossypium hirsutum* L.). In the United States, *Fov* race 1 is recognized as the most common race associated with the disease; however, recent surveys have shown an increase in the distribution of other races. Isolates collected from a cotton field in Lubbock County Texas were identified as races 1, 2 and 3 based on translation elongation factor (EF-1α). Genotypic and pathogenic differences have been documented among races; however, little information relative biological differences is available. Growth chamber studies were conducted to determine the influence of temperature on hyphal growth. Petri dishes containing 20 ml of quarter-strength potato dextrose agar (¼PDA) were inoculated with 0.5-cm diam. ¼PDA plugs obtained from the growing margin of 3-day-old *Fov* cultures. Growth chambers were maintained at 5, 10, 15, 20, 25, 30, 35 or 40°C. Isolates were arranged in a randomized complete block with 10 replications and the experiment was repeated once. Hyphal growth was measured in four directions from the center of each plug 24, 48, 72, 96 and 120 hours after inoculation. Daily growth rates were greatest for TX-29 (race 3), followed by TX-36 (race 2), and TX-31 (race 1) at 15, 20 and 25°C averaging 0.30, 0.41 and 0.57 cm day⁻¹, respectively. Hyphal growth was similar for the three isolates at 30°C and was completely suppressed at 40°C. Although subtle, these differences suggest that temperature can affect growth of *Fov*, and warmer soil temperatures experienced over the past several growing seasons may be attributed to the increased distribution of different *Fov* races.

Field performance of cotton varieties in response to root-knot nematode (*Meloidogyne incognita*)

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Phytopathology 104(Suppl. 2):S2.11

The Southern root-knot nematode (*Meloidogyne incognita*) is an important pest of cotton (*Gossypium hirsutum* L.) throughout cotton producing areas of the United States. A lack of chemical management options has led to a renewed interest in developing varieties with improved resistance. Studies were conducted from 2011 to 2013 in fields naturally infested with *M. incognita* to compare nematode reproduction and lint yields among commercially available cotton varieties and advanced breeding lines. Nematode reproduction (eggs plus second stage juveniles) varied by year and location; however, differences were observed among genotypes. Reproduction was lower for standard varieties with partial resistance, such as 'DP 174RF', 'Phy 367WRF' and 'ST 5458B2F', when compared to susceptible standards, such as 'DP 1044B2RD' or 'FM 9160B2F' and 'Phy 499WRF'. In addition, newer varieties such as 'FM 2011GT' and 'ST 4946GLB2', as well as several advanced breeding lines, supported lower reproduction than the commercial standards. Environmental conditions differed between growing seasons, thus affecting yields. Lint yields for all of the partially resistant varieties were greater than those of the respective susceptible varieties. Furthermore, yield for the advanced breeding lines were similar to those for the partially resistant varieties. These results demonstrate the importance of using partially resistant varieties to manage root-knot nematodes, and that several advanced breeding lines possess attributes similar to commercially available partially resistant varieties. Additional research of these genotypes and others under a broad range of nematode pressure and environmental conditions is needed.