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First report of *Tobacco ringspot virus* associated with decline symptoms of an American hybrid grape cultivar in Texas

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During surveys for grapevine viruses in Texas vineyards, Blanc du Bois vines showing symptoms consistent with those described for grapevine-infecting nepoviruses were sampled in a vineyard block in South Texas. Total nucleic acid extracts from four symptomatic and four nonsymptomatic vines were subjected to a two-step reverse transcription (RT)-PCR using published diagnostic primers targeting *Grapevine fanleaf virus* and *Tobacco ringspot virus* (TRSV). Only TRSV-specific bands were obtained from the symptomatic vines. Molecular typing of TRSV isolates in two symptomatic vines was accomplished with primer pairs TRSVRdRpfwd6005/TRSVRdRprev7067 and TRSVCPfwd2442/TRSVCPprev3452 which amplified 1,055 bp RNA-dependent RNA polymerase (RdRP) and 1,013 bp coat protein (CP) gene-specific DNA bands from both vines. In pairwise comparisons of sequences obtained from cloned amplicons, the derived partial RdRP cistron (KY304030-35) showed 92-100% nucleotide (nt) & 97-100% amino acid (aa) identities among themselves but shared 88-96% nt & 94-97% aa identities with corresponding GenBank sequences of TRSV. The obtained partial CP cistron (KY304036-40) showed 100% nt & 99-100% aa identities among themselves but shared 92-97% nt & 98-100% aa identities with corresponding GenBank sequences of TRSV. To our knowledge, this is the first report of TRSV infecting grapevines in Texas and the first record of Blanc du Bois, an American hybrid grape, as host to the virus.

Survey for Grapevine Leafroll Associated Virus 3 in Texas Vineyards

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Grapevine leafroll disease (GLD) is distributed worldwide and is a major, damaging disease of winegrapes (*Vitis vinifera*). The most ubiquitous and economically important among viruses associated with GLD is *Grapevine leafroll associated virus 3* (GRLaV-3). This virus was previously confirmed in one Texas vineyard in 2014, but little is known about the impact on winegrape production within the thriving industry in the state. A pilot survey was initiated in 2016 to learn how widespread GRLaV-3 is throughout the major Texas winegrape growing regions. Prospective vineyards were identified through grower contacts and viticulture advisors based on a suspected history of virus problems. Petiole samples were collected from May to September, 2016, and processed using ELISA and RT-PCR for presence of the virus. A total of 132 grapevines were sampled from 23 vineyards distributed throughout 15 counties. Forty-three grapevines located in 15 different vineyards in 6 of the surveyed counties tested positive for GRLaV-3. The vineyard locations ranged from the northernmost county (Terry) to the southernmost county (Hidalgo), and represented many of the most important AVAs in Texas. A total of 12 varieties of grapes, including both French and French x American varieties were found to be infected. This

survey has proven extremely useful in establishing trends in symptoms, vine health, and geographic distribution for GRLaV-3 in Texas. In addition, the information obtained will provide guidelines for further survey during 2017.

Mississippi State University Plant Diagnostic Lab annual activity report for the Southern Plant Diagnostic Network

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Between July 1, 2015, and October 31, 2016, the Mississippi State University Extension Service Plant Diagnostic Lab (MSU-PDL) uploaded 1,184 plant disease diagnoses to the National Plant Diagnostic Network (NPDN) national repository. Fifty-six of those diagnoses were uploaded to the national repository for the state of Mississippi for the first time. The most significant of the 56 first entries was a previously undescribed stem canker disease of interspecific hybrid impatiens caused by the fungus *Macrophomina phaseolina*. The stem canker disease was published as a Disease Note in Plant Disease Volume 100. Digital camera systems were updated for 3 microscopes in the MSU-PDL. The plant diagnostic lab contracted the University of Georgia Consortium for Internet Imaging and Database Systems (CIIDS) to customize a web application for diagnostics and laboratory management. The diagnostician attended one training, one workshop, six regional or national conferences, one webinar, participated in 17 NPDN conference calls, served as chair of the NPDN Diagnostics Program Area Committee, and gave nine NPDN-related presentations to local and national audiences.

State Report for Kentucky: Accomplishments and Outreach from the University of Kentucky Plant Disease Diagnostic Laboratory

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There are two branches of the University of Kentucky Plant Disease Diagnostic Laboratory (UK-PDDL) that process routine diagnostic samples, participate in surveys for regulated pathogens and provide plant disease educational resources for County Extension personnel and the general public. Disease problems of new crops or novel production systems are often first observed on samples submitted to the PDDL for diagnosis. Thus diagnostic samples become the basis of applied research initiatives at UK, which expands the PDDL's impact from the needs of an individual grower to multiple growers throughout the state and region. In 2016, a number of new or unusual diseases were investigated, including increasing incidence of bacterial and fungal foliar diseases of tobacco, diseases of hemp as a new crop in the state, and diseases of vegetables produced in greenhouse and high tunnel systems. Hands-on training sessions in disease diagnosis, disease management and using available resources (e.g., UK-PDDL) were conducted for County Extension Agents in western and central/eastern Kentucky during the growing season.

Management of Plant-parasitic Nematodes in Soybean Using Site-specific Methods

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Southern root-knot nematode, *Meloidogyne incognita*, is responsible for substantial yield losses on soybean grown in the Mississippi River Delta. Evidence suggests seed treatment nematicides, fluopyram and abamectin, have decreased reproduction and root galling by root-knot nematodes (RKN) but yield protection in fields with higher nematode pressure is unclear. The objective of this work was to determine the efficacy of these seed treatment nematicides compared to 1,3-dichloropropene (1,3-D) applied site-specifically. In a field near Backgate, AR, three strips of 1, 3-D were applied with a coultter rig three weeks prior to planting, 16 rows wide, on 96.52 cm raised beds that extended the length of the field. Armor DK 4744 soybeans without nematicide, fluopyram, abamectin, or no nematicide seed treatment within 1,3-D strips were planted in adjacent strips of equal size, replicated three times. Data was collected at ten fixed locations within each strip and yield data provided by a yield monitor. Populations of RKN were significantly less at harvest in the 1,3-D strips than fluopyram, and strips lacking nematicide ($P=0.05$). Yield was significantly greater in the 1,3-D strips than all other treatments while fluopyram and abamectin were not different than strips lacking nematicide ($P=0.001$). The distribution of RKN at harvest was uniform ($P=0.08$) suggesting 1,3-D would be economically beneficial as a whole-field application when a susceptible soybean is planted.

Resistance-proof approaches to suppression of strawberry powdery mildew (*Podosphaera aphanis*)

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Annual production systems for strawberry involve growth of nursery plants in geographic regions and under management programs distinctly separated from commercial fields. Actions in the nursery can have profound downstream effects in fruit production. To break the link between nurseries and commercial fields, we are exploring two methods to suppress Strawberry Powdery Mildew (SPM) that bypass selection for fungicide resistance. Sulfur is widely used against many powdery mildews, but is often avoided due to anecdotal

reports of phytotoxicity at high temperatures. In both high tunnel and open field trials, we applied wettable and flowable sulfur formulations at rates up to double the maximum labeled concentration to cultivar 'Florida 127' without phytotoxicity, despite frequent occurrence of temperatures above 30 C. Efficacy of sulfur treatments approached that of highly effective synthetic fungicides (quinoxifen and cyflufenamid). Water applied in pulses 1min/day and 1min/4xday also significantly suppressed SPM compared to untreated controls. Severity of SPM was universally higher in high tunnels than in open fields across all treatments. Pulsed misting with water and sulfur sprays may represent useful augmentations of an integrated program of SPM suppression in strawberry nurseries and fruit production fields that directly reduces risks of degraded performance of valuable synthetic fungicides due to selection for resistance.

Assessing virulence and phylogenesis of QoI-resistant and -sensitive *Cercospora sojina* isolates from Mississippi soybean

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Frogeye leaf spot is a foliar soybean disease caused by *Cercospora sojina* Hara, which until recently was successfully managed with quinone outside inhibitor (QoI) fungicides. However, widespread QoI resistance in 95% of the Mississippi soybean production area was recently reported. For the purposes of the current research, a subset from the 2013-14 *C. sojina* collections which included, 13 QoI-resistant isolates exhibiting the G143A substitution and 11 QoI-sensitive isolates without the substitution were used. Greenhouse studies were conducted with the QoI-resistant and -sensitive *C. sojina* isolates to determine whether the presence of the G143A substitution influenced virulence. Soybean seedlings were inoculated with conidial suspensions (5×10^4 conidia/ml) of each isolate pre and post 140 day subculture. Virulence, assessed as percent area of leaf necrosis was determined 21 days post-inoculation. In addition, a five loci phylogenetic analysis assessed the relatedness of QoI-resistant and -sensitive isolates. No significant differences in virulence were noted between QoI-resistant and -sensitive isolates pre and post 140 day subcultures indicating no fitness cost in virulence associated with the G143A substitution. The phylogenetic analysis showed no differences in relatedness among *C. sojina* isolates regardless of fungicide sensitivity. Results of these studies indicate a fitness cost is not associated with QoI-resistant *C. sojina* isolates in Mississippi soybean.

Refining estimates of the distribution of plant-associated fungi using host-fungus association and plant distribution data

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There are approximately 2.3 million specimens of microfungi preserved in North American institutions. Yet, accurate information on their geographic distribution is frequently lacking. But many of these species are plant-associated fungi and information produced by the digitization efforts of natural history collections and existing databases with information on host-fungus associations and plant species range provides the means to estimate the distribution of plant-associated fungi in North America. We set out to develop an application that aggregates information from disparate sources to provide a means to estimate the distribution of plant-associated fungi which can be used to test hypotheses about host specificity and/or develop a targeted approach to fill in knowledge gaps about the occurrence of individual fungal species. Our application, mGHost, requires fungal taxa as input and currently integrates information from IndexFungorum, the USDA Fungus-Host Distribution database, and the LSU herbarium. mGHost builds a database containing the assimilated metadata (e.g. host locality, habitat) and produces a distribution map of associated host species when GPS coordinates are available. Future versions of mGHost will integrate additional institutional collections, MycoPortal, and the USDA Plants database to provide more detailed distribution estimates and expand output to include data for downstream analyses, such as ecological niche modeling.

Pathogen Triggered Plant Volatiles Induce Systemic Susceptibility in Neighboring Plants

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To protect against herbivory by insects, plants initiate a multifaceted defense response. In contrast to direct defense, characterized by the production of toxic metabolites, indirect defense relies on volatile emissions to attract predators of the insect herbivores and warning neighboring plants to prepare for attack. These volatiles are known as herbivore-induced plant volatiles (HIPVs). One subgroup of HIPVs is Green Leaf Volatiles (GLVs), which govern plant-plant and plant-insect communication. Despite the strategic role of GLVs in insect defense, their function in pathogen defense has been overlooked. Herbivory induced GLVs prime neighboring plants against impending insect attack by enabling them to produce greater levels of Jasmonic acid (JA). This phenomenon is called induced systemic resistance (ISR). Unexpectedly, pathogen-induced plant volatiles (PIPVs) induce systemic susceptibility (ISS) in neighboring plants, rather than ISR, as plants exposed to PIPVs become more susceptible to *C. graminicola*. Susceptibility in neighboring plants is caused by a release of GLVs from infected plants that induces JA production in neighboring plants. JA is a susceptibility factor for *C. graminicola*. LOX5, a GLV inducible gene, is required to signal the production of JA and is induced in plants exposed to PIPVs. Taken together this research demonstrates a model for epidemics in the field based on infected plants priming neighboring plants for future infection.

***Rhizoctonia solani* field phenotyping in a peanut RIL population and lab inoculation method**

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Limb rot, caused by *Rhizoctonia solani*, is an important disease of peanut. In 2016, 128 lines from a RIL population derived from Tifrunner X NC3033 and 8 standard lines were evaluated for susceptibility to limb rot. The lines were planted in randomized complete blocks with two replicates. The field was fumigated with 100% chloropicrin (300 lb/A) under plastic before planting. In mid-season, the plots were infested with oat seeds colonized by *R. solani*, and watered for 3 consecutive days. At harvest, inverted plants were rated based on the percentage of infection. Disease severity ranged from 0 to 27.5%, and NC3033 was more resistant than Tifrunner (10.5 and 16.0%, respectively). There was evidence of transgressive segregation of resistance in some RIL's. While the field phenotyping presents good data for resistance mapping, developing a good lab inoculation method is also our concern. Stem inoculations with PDA plugs of *Rhizoctonia* mycelium and incubated at 16, 20 or 24°C resulted in 10%, 60% and 50% of the stems infected, respectively. With 3 PDA plugs per stem, at 20°C and 95% humidity, 85% of the stems were infected after 1 week. Similar methods of leaf inoculation have resulted in 100% infection. The correlation between lab and field assays has yet to be determined.

A Risk Assessment Model for Bacterial Leaf Spot of Pepper Based on Concentrations of Macronutrients and Micronutrients and Micronutrient Ratios

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Regression models (2012-2014) based on macronutrients, micronutrients and micronutrient ratios in pepper tissues and bacterial leaf spot (BLS) severity were developed. Factors used to select a model for validation included highly significant *P* values, high adjusted *R*² values, and low variance inflation factor (VIF) values (<5). In addition, salicylic acid (SA) concentrations and relative expression of non-expressor pathogenesis-related gene1 (*NPRI*) in pepper tissues were also considered in model selection. One model consisting of concentrations of copper (Cu), manganese (Mn) and potassium (K) and the iron to zinc ratio (Fe:Zn) as independent variables was used for validation experiments in three different Georgia counties in 2015. When pooled AUDPC values for BLS epidemics from the three locations were plotted against model predictions, there was a significant fit (*P* < 0.0001) with a *R*²=0.95. Relative gene expression of both *NPRI* and *PR1* genes were significantly (*P* < 0.01) higher in peppers grown in predicted low risk sites compared to peppers from high risk sites in Colquitt and Worth counties. A similar trend was observed in Tift County where expression of *NPRI* in peppers from low risk areas were approximately 40-fold higher than in peppers from high risk sites. We also observed that mean *MnSOD* gene expression was significantly higher in peppers from a low risk site (fold change = 3347) compared to high risk site (fold change = 59) (*P* = 0.055). Although BLS severity will fluctuate depending on environmental conditions, the data indicate that the level of risk at a particular location may be influenced by how macronutrient and micronutrient concentrations affect plant disease resistance genes in the SAR pathway.

New and Emerging Diseases in Puerto Rico in 2016

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The University of Puerto Rico Plant Diagnostic Clinic (PRPDC) has been conducting the identification and management of pests and diseases since 2005. In 2016, the PRPDC processed approximately 1,087 samples. Prevalent conditions in 2016 including high temperatures and humidity favored fungal pathogens including *Corynespora cassiicola* which was confirmed in various crops including tomato, pepper and yam (*Discorea alata*) in the area of San German and San Lorenzo. Detection of *Clavibacter michiganensis* pv. *michiganensis* from submitted tomato and pepper plant samples was confirmed by serology and pathogenicity tests. Tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annuum*) infected plants became systemically wilted and developed a canker in the lower stem. Bacterial wilt caused by *Ralstonia solanacearum* was identified in *Capsicum chinense* grown in a field in Yabucoa, which is the first report for sweet chili pepper in Puerto Rico. The disease was also observed in Lajas and Coamo affecting isolated plants. *Ralstonia solanacearum* was identified through isolation on nutrient agar (NA), semi-selective triphenyl tetrazolium chloride agar (CTZ), DAS ELISA (Pathoscreen ® Kit, Agdia, Indiana, U.S.A.) and polymerase chain reaction (PCR). This disease has been identified previously affecting tomatoes in Puerto Rico. Early identification of these diseases is essential to prevent their spread to other hosts.

Effects of Glyphosate on Soybean Nutrition, Endophytic Colonization by *Cercospora* cf. *flagellaris* and Development of *Cercospora* Leaf Blight

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Since the introduction of glyphosate-resistant (GR) crops, *Cercospora* leaf blight (CLB) has become one of the most prevalent foliar soybean diseases in Louisiana. Over the past decade, glyphosate has been reported to have effects on plant mineral nutrition and diseases in certain hosts. In order to test the hypothesis that glyphosate affects soybean mineral nutrition and predisposes plants to CLB, six GR soybean varieties were either not treated or treated with glyphosate in field experiments at three locations in Louisiana from 2014 to 2016. Plants were evaluated for leaf concentrations of 13 nutrients, foliar disease symptoms, and biomass of *Cercospora* cf.

flagellaris assessed with real-time PCR for the CTB6 gene of *C. cf. flagellaris*. The effects of glyphosate on leaf concentrations of Al, Fe, Mn, N, Na, and K were location-dependent. Glyphosate increased uptake of Zn in all experiments. Biomass of *C. cf. flagellaris* was consistently detected at higher concentrations in glyphosate-treated samples. Enhanced uptake of Zn, a key component of a transcriptional activator (CTB8) in the cercosporin biosynthetic pathway, may be associated with higher concentrations of DNA of *C. cf. flagellaris* in glyphosate-treated plants. However, severity of CLB purple leaf symptom was significantly lower in glyphosate-treated plots compared to controls. These results suggest that glyphosate affects colonization by *C. cf. flagellaris* during the endophytic stage of pathogen development.

LOX10-mediated signaling increases maize susceptibility to *Colletotrichum graminicola* by suppressing SA biosynthesis

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Green leaf volatiles (GLVs) are an important class of lipoxygenase (LOX)-derived, stress-inducible molecules that are paramount in mediating plant-pathogen interactions. GLVs modulate plant defenses by inducing biosynthesis of the phytohormone, jasmonic acid (JA). GLV-mediated activation of the JA pathway results in substantial defense against insects and necrotrophic pathogens, leading to the pervasive view that GLVs *only* increase resistance to pathogens. Contrary to this view, we show that the GLV-deficient maize lipoxygenase mutant, *lox10*, has enhanced resistance against anthracnose stalk rot (ASR) and leaf blight (ALB) caused by *Colletotrichum graminicola*. To investigate the underlying reasons for LOX10-mediated susceptibility to these pathogens, phytohormone and metabolite analysis was conducted in wild-type (WT) and *lox10* mutant stalks inoculated with *C. graminicola*. Results revealed that *lox10* mutants have increased levels of salicylic acid (SA), a well known phytohormone that functions in defense against hemibiotrophic pathogens.

Virginia Tech Plant Disease Clinic Highlights--2016

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The Virginia Tech Plant Disease Clinic (PDC) provided 1665 diagnoses, identifications and control recommendations for a total of 1309 plant samples, in addition to replies to 208 electronic and phone inquiries, in 2016. An unusually large number of samples (214) were boxwoods, of which 32 were positive for boxwood blight. The Clinic also received samples of dying *Rhododendron maximum* from forested areas exhibiting widespread plant mortality. The VT Plant Disease Clinic conducts annual surveys to collect data regarding client satisfaction. The 2016 survey results indicated that commercial growers agreed that PDC services increased their crop quality (79%), allowed them to develop a more strategic integrated pest management approach (71%) and increased profitability (64%). Eighty-one percent of non-commercial growers agreed that their understanding of plant disease and/or abiotic problems was increased by PDC services and respondents agreed that PDC services ensured they only use appropriate pesticides (54%), only use pesticides when they will be effective (57%) and only use pesticides when there is a serious threat to the health of the plant (58%).

WHAT about Social Media? Communicating Science in the Digital World

Tracy Hawk, Mandy Little and Kevin Ong, Texas A&M AgriLife Extension Service, College Station, TX

Communicating science effectively to the general public is a challenge that we, as plant pathologists, faces each day. This seemingly mundane and yet crucial activity is necessary to share our passion and love of plant diseases and plant pathogens, to capture the interest of the general public. The Texas Plant Disease Diagnostic Lab (TPDDL) is a part of the Texas A&M AgriLife Extension Service which provides diagnostic services to the people of Texas and beyond. TPDDL also develops and distributes plant disease factsheets, plans and executes public outreach events and provides plant health educational support to county agents and other AgriLife personnel. In the past few years, social media has been a “big” technological trend. In many academic institutions, the use of social media is encouraged to reach the masses. The TPDDL uses several social media (facebook, twitter, Instagram) outlets to promote our activities and to share our knowledge. One of our more popular product is the “What Wednesday” vlog (video-log). The goal of each video is to identify plant health related situations that are relevant to the people of Texas and to share tidbits of plant health related knowledge (symptom recognition and treatment recommendations). Our experience has made us aware that the effective outreach social media tool, such as our “What Wednesday” vlog, does not have to be elaborate and costly, but can be rapidly produced in a simple and inexpensive manner.

Management of root-knot nematodes in the northern Delta using Telone II soil fumigant and root-knot nematode resistant cotton varieties

Robert A. Haygood, Dow AgroSciences LLC, Memphis, TN, Mustafa McPherson, Phytogen Seed Company, Leland, MS and Andrea Jones, University of Missouri, Portageville, MO

Telone® II soil fumigant is used to control root-knot nematode (RKN), *Meloidogyne incognita*, and other plant parasitic nematodes in cotton. The RKN occurs in most cotton growing areas and thrives in sandier type soils. Cotton yield losses from RKN often exceed 20%. PHY367WRF, which expresses moderate resistance to RKN, was introduced by Phytogen Seed Company LLC in 2010 and was followed by the release of PHY427WRF in 2014 which has the highest known level of resistance to RKN in cotton. These new early/mid maturity varieties were compared to the susceptible varieties PHY375WRF and PHY499WRF with and without Telone II in southern MO and in northern areas of MS and AR in 2013 and 2014. All fields had sandier type soils with predominant RKN

populations. Yield increases of 8%, 12% and 30% were achieved when Telone II was applied prior to planting PHY427WRF, PHY367WRF and the susceptible varieties, respectively. As compared to the susceptible varieties, PHY427WRF suppressed RKN reproduction by ~90% as indicated by soil nematode counts at the end of the season. RKN resistant varieties offer no control of other nematodes such as reniform, *Rotylenchulus reniformis*, thus soils infested with multiple species must be managed with appropriate control practices, which could include the use of RKN resistant varieties and site specific applications of Telone II.

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Improved diagnostic methods for *Xylella fastidiosa* infecting pecan and related *Carya* species

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Pecan bacterial leaf scorch (PBLs) is a chronic disease caused by *Xylella fastidiosa* subsp. *multiplex* which can lead to yield losses in susceptible varieties. Symptom development begins with necrosis of leaflet tips and margins, and later progresses toward the leaf base and midribs in a uniform pattern. Pecan samples manifesting symptoms of PBLs have been observed by the USDA-ARS Pecan Breeding & Genetics Program in Texas. However, inconsistencies in diagnostic results revealed the need for improved detection methods for *X. fastidiosa*, based on enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). We screened 126 geographically diverse accessions from NCGR-*Carya* collections in Texas, 11 samples from commercial pecan orchards in Medina and Uvalde counties, Texas, and 4 samples from wild *Carya* species in Daviess County, Indiana. Sap was extracted from symptomatic and asymptomatic shoots using a Scholander-type pressure bomb and tested by ELISA. PCR using previously-reported *Xylella*-specific primer sets was compared with the ELISA results. The majority of *Carya* samples from Texas and Indiana were found to be positive for the presence of *X. fastidiosa* by both ELISA and PCR methods. A panel of twenty geographically distinct *Carya* samples were selected for sequencing of the conserved hypothetical protein, HL. Sequences were subjected to an alignment by BLASTn (NCBI) and found to have a 98% identity to *X. fastidiosa*.

Using unmanned aircraft vehicles in rice cropping systems

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A small unmanned aircraft system (UAS) now offers tremendous opportunities for use in the agricultural sector. With the use of rotary winged UAS equipped with a traditional RGB or a multispectral imaging camera, we obtained aerial images of rice plots at the Texas A&M Rice Research Center in Beaumont and commercial rice fields in Texas. This presentation will deliver a succinct background and basic operation procedure on the use of UAS in monitoring rice cropping systems from pre-flight planning to post-flight image processing. We will discuss the current capabilities of our UAS in terms of autonomous flight, the technical uses of onboard RGB and multispectral imagery, the latest software capabilities using UAS-based aerial images in measuring distances, photogrammetric triangulation for image stitching, and georeferenced orthomosaic export. In the near future, the UAS applications will facilitate crop surveying, plant condition assessment by NDVI, biomass or yield estimation, and management of weed, insect and disease problems in rice cropping systems.

An update on the University of Georgia (UGA) Extension Plant Pathology Southern Plant Diagnostic Network (SPDN) Plant Disease Clinics

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The department of plant pathology at UGA maintains extension plant disease clinics in Athens and Tifton as part of the Southern Plant Diagnostic Network. The clinic diagnosticians and faculty extension specialists aid county extension faculty and clientele (arborists, golf courses, growers, homeowners, researchers and retailers) in diagnosing plant disease problems and providing information on disease management. The Tifton clinic analyzes corn, cotton, pecan, soybean, tobacco and commercial vegetables, while the Athens clinic analyzes forage, fruit, homeowner, ornamental, small grain, turf, tree and wood rot samples. Since 2003, over 23,500 commercial and homeowner physical plant pathology samples were processed in the clinics. Physical and digital sample information is stored in the web-based Distance Diagnostics through Digital Imaging (DDDI) database. Between 2014 and 2016, the largest number of physical samples processed were in the vegetable crop category. Among the diagnoses provided, there were several first finds in the state, including boxwood blight (*Calonectria pseudonaviculata*), boxwood dieback (*Colletotrichum theobromicola*), cucurbit yellow stunt disorder virus, *Pseudomonas* and *Xanthomonas* leaf spots in cucurbits, tomato pith necrosis (*Pseudomonas corrugata*) and yellow bud in onion (*Pseudomonas coronafaciens*). This program is committed to providing accurate, unbiased diagnoses and recommendations to facilitate timely and effective management.

Refining Supplemental Chloropicrin Applications in Large Scale Plots

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Since the transition from methyl bromide to current fumigants, growers have had a resurgence of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) on tomato. Conventional fumigation practices leave roots exposed to pathogens in under-treated areas of the bed. Supplemental chloropicrin (Pic) at 150 lb/A applied along the bed edges with a Yetter Avenger Coulter system was shown to treat soil along bed edges and row middle. The goal was to conduct two large-scale demonstration trials to evaluate the depth of supplemental Pic application, and reduced in-bed application rates of Pic-Clor 80 when using the supplemental Pic approach. In the first trial, Pic was applied at a 4, 6.5, and 9.5 inch depth, in combination with and without in-bed fumigation. No Significant differences were noted in the levels of Fusarium recovered after fumigation between Yetter depths. Incidence, however was significantly increased in treatments with 4 and 6.5 depth without in-bed fumigation compared to 4 inch depth with fumigation which had the lowest number of symptomatic plants. The second trial examined in-bed rates of Pic at 200, 180, 160, and 140 lbs/A in conjuncture with and without the supplemental Pic. Plant heights, incidence ratings, and yields were taken throughout the season. Plots receiving the supplemental Pic with a in-bed fumigation rate of 140 lb/A yielded 35% more at the first harvest than plots receiving the highest in-bed fumigation rate of 200 lb/A without the supplemental Pic.

2015-2016 Diagnostic Developments in the UF-IFAS Plant Diagnostic Center

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The UF-IFAS Plant Diagnostic Center serves as the hub lab for the SPDN and the FPDN, processing an average of 3,000 samples for routine diagnostics, regulated plant pathogens, and special projects each year. In 2015-2016 the PDC processed samples from 20 states and 21 countries. Fungi and oomycetes continue to represent about 85% of diagnosed pathogens, and turf, woody ornamentals, vegetables, and small fruit samples dominate. Palm samples have steadily increased over the past two years and, along with turf, make up a significant portion of both domestic and international samples. Blueberry plants and St. Augustine turf continue to be well-represented in samples overall, and both hosts have been recently afflicted with new diseases in Florida. The lab staff continue to participate in NPLAPP certification for HLB and *P. ramorum* molecular diagnostics, and sends staff to new APHIS-NPDN (Beltsville) trainings. APHIS is in the process of evaluating our planned deviation for HLB qPCR detection on an ABI Flex 6 system, and we plan to do another for *P. ramorum* detection in 2017, thanks to the post-doc in our trial one-year applied post-doctoral diagnostician position. We will be looking to add a diagnostician to our staff summer 2017 to assist with regional engagement, lab accreditation, phytosanitary testing, and routine diagnostics.

Fungicide sensitivity of *Corynespora cassiicola* (target spot) isolates

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Corynespora cassiicola is the causal agent of target spot of cotton. Fungicides are used to control target spot. Here, fungicides from three classes and at varying concentrations were assessed to determine effects on mycelial growth and spore germination of *C. cassiicola*. Objectives were to determine the sensitivity of isolates of *C. cassiicola* from cotton to metconazole, fluxapyroxad and pyraclostrobin and to compare the sensitivity to isolates from other hosts. Forty total isolates from five states (GA, AL, FL, LA, TN) were collected from cotton and 20 isolates were collected from other hosts (soybean, hydrangea, cucumber, pepper, tomato). Fungicide concentrations varied from 0 to 3 µg/mL. Sensitivity of *C. cassiicola* to metconazole and to fluxapyroxad was determined using a mycelial growth assay. Sensitivity of isolates to pyraclostrobin was determined using a spore germination assay. Each isolate was tested to determine the fungicide concentration at which mycelial growth or spore germination was inhibited by 50% (EC50). EC50 values for metconazole ranged from 0.015 to 0.203 µg/mL with a mean value of 0.052 µg/mL. EC50 values of metconazole for isolates of *C. cassiicola* from other hosts ranged from 0.01 to 0.976 µg/mL with a mean value of 0.04 µg/mL. From this study, EC50 values varied among isolates; however the mean EC50 values from cotton and other hosts were similar. The range of EC50 values from crops other than cotton was nearly five times that of isolates from cotton. This may be the result of more limited exposure of cotton isolates to fungicides.

An Evaluation of QoI and SDHI Fungicide Sensitivity to Target Spot of Tomato in Florida

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Target spot of tomato caused by *Corynespora cassiicola* is one of the most damaging foliar and fruit diseases in Florida. In the last 2 years, 95 isolates were collected from 7 tomato producing counties in Florida and evaluated for sensitivity to 2 quinone outside inhibitor (QoI) and 5 succinate dehydrogenase inhibitor (SDHI) fungicides using a mycelial growth inhibition assay. Initial assays screened a representative set of 32 isolates across 9 fungicide rates to calculate an effective concentration to inhibit mycelial growth by 50% (EC50) and to identify a discriminatory concentration. Over 90% of the *C. cassiicola* isolates collected were resistant to the QoI fungicides azoxystrobin and fenamidone, exhibiting EC50 values in excess of 100 ppm in the presence of SHAM. Sensitivity of the initial 32 isolates to SDHI fungicides varied and was classified as either sensitive (EC50 < 3 ppm), moderately resistant (EC50 = 3-10

ppm), or resistant ($EC_{50} > 10$ ppm). Based on a 5 ppm discriminatory dose, all 95 isolates were evaluated for insensitivity against the SDHI fungicides, boscalid (73.2%), penthiopyrad (57.7%), benzovindiflupyr (59.1%), fluxapyroxad (30.4%), and fluopyram (7.2%). Individual isolates varied in their level of resistance and cross-resistance among the SDHI fungicides. The study of these representative isolates and their relationships can progress the understanding of SDHI resistance development in Florida.

Quantifying the impacts of fungicide phytotoxicity in Mississippi soybean

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Following the widespread detection of quinone outside inhibitor (QoI; FRAC code 11) resistant frogeye leaf spot (FLS) (*Cercospora sojina* Hara) in Mississippi, soybean farmers have had to rely on additional modes of action (MOA) to effectively manage FLS. One issue to applying fungicides that may contain demethylation inhibitors (DMI; FRAC code 3), can be phytotoxicity on treated soybean leaves. Field trials were conducted in 2015 and 2016 to assess fungicide efficacy and phytotoxicity on two soybean cultivars with differing resistance to FLS on QoI-resistant FLS. The fungicides chosen are known to produce moderate to severe phytotoxicity. Seven treatments, including six fungicides comprised of single or multiple MOAs (FRAC codes 3, 7, 11, U12), were applied at R2, R3, and R4 in addition to a non-treated. FLS and phytotoxicity were visually assessed pre- and several times post-application. Plant height, number of pods and nodes were recorded to determine physiological impacts. MOAs within FRAC code 11 and 3 significantly reduced FLS severity compared to the non-treated. In addition, products containing FRAC code 3, and more specifically prothioconazole, resulted in significantly greater phytotoxicity than the non-treated. Additionally, plant heights and number of nodes per plant were not significantly different between fungicides and the non-treated. More importantly, yield was not significantly reduced as a result of fungicide phytotoxicity.

Utilizing global positioning and geographical information systems for tracking Phymatotrichopsis root rot disease in alfalfa

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Phymatotrichopsis omnivora is the causal agent of Phymatotrichopsis Root Rot (PRR) disease that severely limits alfalfa production in Oklahoma. Alfalfa fields infested with *P. omnivora* can have reduced stand life and productivity. The extent of disease spread occurring in a growing season greatly affects alfalfa stand longevity, but is poorly understood. In 2016, we conducted a study on a 25-hectare PRR infested three-year old alfalfa hay production field at the Noble Foundation's Red River Farm, Burneyville, OK. Twenty five individual actively growing PRR disease rings were randomly selected in June and monitored at two to four week intervals until October. PCR performed on three roots sampled from each disease ring confirmed the presence of *P. omnivora*. A Trimble Geo7x handheld coupled with Zephyr Model 2 antenna was employed to walk along the circumference of each disease ring collecting vertices of disease ring polygons at one-second interval with post-processed accuracy of 2.5 cm. Data from the Geo7x handheld was transferred using GPS Pathfinder and visualized in ArcMap 10.3.1 software. Preliminary results indicated more disease spread occurring during mid-July, increasing towards the west side of the disease ring. The average disease front movement was 3 meters between June and October. Handheld global positioning systems thus offer an invaluable tool in accurately tracking disease progression.

Texas Plant Disease Diagnostic Lab - State Report

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The Texas Plant Disease Diagnostic Lab (TPDDL) in College Station, TX is a Southern Plant Diagnostic Network member lab. It is one of the two NPDP member labs in Texas. TPDDL sample load averages 2663 samples annually for the past 5 years. In 2016, the TPDDL processed over 2800 submitted samples. Analysis of the sources and composition of these submitted samples provides a generalized view of plant health issues affecting Texas. Majority of samples processed were in support of Texas Department of Agriculture regulatory inspection activities (28%). This is followed by samples submitted from commercial landscape maintenance industry (14%) and commercial nursery and greenhouse producers (13%). When categorized by crop type, tree fruit accounted for 40% of all samples. Ornamentals (woody and herbaceous) and shade trees accounted for 19% and 15%, respectively. We believe that unusually wet conditions and relatively mild temperatures contributed to a large number of samples were diagnosed with diseases caused by *Phytophthora* sp. on multiple types of plants. Notable TPDDL highlights include the diagnosis of bacterial blight causing growing issues on leafy green vegetables in the Winter Garden region, and providing informational and educational support for citrus diseases (citrus greening and citrus canker) in affected or adjacent counties to plant quarantines zones in the greater Houston area.

A tale of two regions: Comparing genotypic and phenotypic profiles of *Stagonosporopsis* spp. in Florida and China

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Stagonosporopsis spp., the causal agents of gummy stem blight, are major pathogens on melon and watermelon. The lack of resistant varieties has led to reliance on fungicides as the main management strategy; however fungicide resistance has been reported. The purpose of this study was to compare fungicide sensitivity profiles of isolates from two major fresh-market cucurbit production regions.

The regions consisted of four provinces in east China (N=69) and 10 counties in Florida (N=90). Isolates were evaluated for sensitivity to the fungicides azoxystrobin, boscalid, tebuconazole, and thiophanate-methyl at dosage rates of 0.096mg/l, 0.034mg/l, 0.128mg/l and 100mg/l respectively. 95 isolates (consisting of 50 isolates from Florida and 45 from China) were genotyped using 20 previously published microsatellite markers. A high Simpson's index (>0.95) and moderate allelic diversity (Hexp=0.478) was observed which indicates high genetic diversity within the isolates surveyed. Fungicide resistance was detected in isolates from both continents with a majority of isolates from China (>90%) and Florida (>75%) exhibiting some level of resistance to boscalid and thiophanate-methyl. A significant percentage of isolates (67%) were also resistant to azoxystrobin in Florida. These results suggest that both regions face similar management challenges related to fungicide usage and that an integrated management program is needed to control this disease in both regions.

Taproot decline: First report of a previously unknown disease of soybean

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Over the past 10 to 15 years, a soybean malady has emerged in the southern United States exhibiting foliar symptoms that include interveinal chlorosis and necrosis, which appears similar to sudden death syndrome and other important root/crown/stem diseases. In addition to the foliar symptoms, affected plants easily break at the soil line, and excised roots exhibit a blackened appearance with severe lateral root and taproot necrosis. When stems are split longitudinally near the crown, white mycelial growth is evident in the pith. Prior to 2016, the disease has been referred to as the "mystery disease" or improperly diagnosed as black root rot, which is caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris. During 2015, soybean plants exhibiting symptoms of taproot decline near Winnsboro, Louisiana, were removed to the laboratory where the suspected pathogen was isolated and Koch's postulates were completed in the greenhouse. Observations of the disease continue throughout the southern U.S. Even though the disease has been observed for several successive years, reports of yield losses have been infrequent. Moreover, in some isolated cases where taproot decline has been observed to be severe, yield losses on the order of 30% have been recorded. Molecular analyses indicate that the pathogen is most likely a member of the *Xylaria arbuscula* aggregate that has not yet been described.

Potential varietal resistance to taproot decline of soybean

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Taproot decline, a new soybean disease, was recently reported in the southern United States and is a major problem in areas where conservation tillage and soybean monoculture are practiced. Anecdotal evidence during previous growing seasons indicates that there may be differences in susceptibility to the pathogen among soybean varieties. In 2016, a pilot study was conducted at two locations (Winnsboro, LA and Stoneville, MS) utilizing infested millet or infested corn cob grit as inoculum in LA and MS, respectively. Experimental units consisted of two row plots where one row was inoculated. Thirty-two varieties included in the study were chosen arbitrarily from the LSU AgCenter official variety trial seed bank. All two-row plots (variety) were replicated four times in a RCBD. Observations were recorded throughout the growing season post-planting and included: stand counts, plant height and grain yield at physiological maturity. In Louisiana, stand, plant height, and yield were reduced significantly by 36, 23, and 72%, respectively, in inoculated rows compared to non-inoculated rows. Stand vs. height and height vs. yield were positively correlated in the Louisiana location. In Mississippi, stand and plant height were reduced significantly by 90 and 28%, respectively, in inoculated rows compared to non-inoculated rows. More research is needed to determine if resistant soybean varieties are available for producers to effectively manage taproot decline.

Development of primers to simplify detection of phytoplasma causing Lethal Yellowing and Date Palm Lethal Decline

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Lethal Yellowing and Date Palm Lethal Decline (DPLD) are two diseases of palms caused by phytoplasma which are identified as *Candidatus Phytoplasma palmae* 16SrIV-A and 16SrIV-D, respectively. In Texas, there are plant quarantine regulations on these two palm diseases. To date in Texas, DPLD (*syn.* Texas Phoenix Palm Decline) has been found in parts of South Texas and the greater Houston area, but Lethal Yellowing has never been detected. Current detection for these phytoplasmas rely on the nested-PCR methods followed by the use of restriction enzymes to digest the nested-PCR product to differentiate the identity of the pathogen. In order to simplify and to optimize our ability to detect and identify these phytoplasma rapidly, we developed two new specific primer sets: LY-FP/RP (5'-GGTGATAACAAAACGGAATC- 3'/ 5'-CTCGGCAATTTGATAAGAAGGGGA-3') to detect Lethal Yellowing producing an expected product size of 369bp, and DPLD-FP/RP (5'-GGCGACAATAAAACAGAATT-3'/ 5'-ATCAATAATGCCTTGTTCAATCA-3') to detect DPLD producing an expected product size of 489bp. These primer sets were designed to target the variable sequence of the GroEL gene between these two phytoplasmas. Initial test results indicate that these primer sets are effective in detecting Lethal Yellowing (16SrIV-A) or Date Palm Lethal Decline (16SrIV-D) via single tube conventional PCR in a process that can be done rapidly.

Effect of planting date, seed treatment, seed quality, flooding and Pythium resistance to soybean emergence

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Seedling diseases, a significant constraint to soybean production in Arkansas, can be affected by planting date, flooding, seed quality, seed treatment, and cultivar resistance. High and low quality seed of 'Hutcheson' (Pythium susceptible) and 'Archer' (Pythium resistant) were treated with metalaxyl, PCNB +carboxin, fludioxonil, metalaxyl+thiram+carboxin, or untreated and planted at three locations and three planting dates from 2001-2003. Half of each test was flooded at emergence for 24 hr. Stands were determined two and four weeks after planting. In 2003, root and hypocotyl discoloration was determined. Across all locations and planting dates seeds treated with metalaxyl+thiram+carboxin had the greatest stands. Hutcheson seeds treated with metalaxyl alone had similar stands to those treated with metalaxyl+thiram+carboxin suggesting that *Pythium* spp. was a major pathogen group reducing stands. Stands from seed of Archer treated with metalaxyl were not significantly different than the untreated seed at 2 wk suggesting that resistance to *Pythium* spp. was active in the field. At 4 wk, metalaxyl treated stands were lower than seed treated with metalaxyl+thiram+carboxin. Root discoloration was lower with Archer than Hutcheson, but there was not a consistent difference in root infection by *Pythium* spp. between the two cultivars. Flooding and low seed quality reduced stands of both cultivars, but did not interact with seed treatment or cultivar.

Potential interaction of zinc and actigard in tomato spotted wilt virus-nicotiana tabacum pathosystem

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Actigard (an analog of Salicylic acid), is a commercially available plant defense inducer. Although, it is effective in plant disease management, the efficacy can be variable at times and the cause of this unpredictability is unknown. Here, we present a possible cause for the variable results using *Nicotiana tabacum* L. and Tomato spotted wilt virus pathosystem. Field studies (2014 and 2015) showed that plants treated with zinc + actigard had significantly higher levels of tomato spotted wilt (TSW) when compared to plants treated with actigard alone. Tissue from plants treated with zinc and zinc+actigard were sampled (field study; 2016) for transcriptome analysis and relative gene expression for non-pathogenesis related protein 1 (*NPR1*) and pathogenesis related protein 1 (*PR1*) genes was determined. Results from the transcriptome analysis showed an up-regulation in genes (134) associated with increased disease susceptibility and a down-regulation in defense associated genes (331) in plants treated with zinc + actigard when compared to plants treated with actigard alone. Additionally, there was significantly higher expression of *NPR1* and *PR1* genes in plants treated with only actigard when compared to the zinc + actigard. These results indicate a potential interaction between zinc and actigard with regards to product efficacy and plant defense.

Genome analyses of a novel, nematophagous fungus in the genus Brachyphoris provides clues on its potential use as biocontrol

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Plant parasitic nematodes are one of the most destructive pathogens of crop plants worldwide. Discontinued use of harmful chemicals has prompted the search for feasible and effective alternative control strategies. Nematophagous fungi are widely distributed in the environment and have evolved different strategies to parasitize and derive nutrition from nematodes. In this study, a previously characterized, but unnamed nematophagous fungus isolate (designated-TN14) was taxonomically classified and its genome sequence was obtained. Based on taxonomic analyses using ITS1-5.8S-ITS2, the fungus was predicted to form a distinct monophylogenetic clade with the genus *Brachyphoris*, a genus of nematophagous fungi related to *Dactylella* and *Vermispora*. Additionally, we obtained the genome sequence of TN-14 via Pacbio sequencing. The genome was assembled into 412 scaffolds, with 76/178 Kb as scaffold N50/L50, and the largest scaffold was 1.12 Mb. Interestingly, 91% of the assembly was included in scaffolds greater than 50 Kb. Alignment of the reads revealed an estimated genome size of 45.6 Mb, which was slightly larger than genome of *Arthrobotrys oligospora* (40.07 Mb). Gene prediction based on *Arthrobotrys oligospora* resulted in 14,461 gene models. Subsequent analyses of the genome of TN-14 are providing insight into molecular mechanism involved in pathogenicity and the viability of using TN-14 as a potential biocontrol for managing plant parasitic nematodes.

2016 Activities: Plant Disease and Insect Clinic (PDIC), North Carolina State University

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NC State's PDIC diagnosed 2671 samples in 2016. Plant pathogens (including nematodes) accounted for 53% of the samples diagnosed; arthropods and abiotic problems accounted for 14% each. Other samples included plants, weeds, and mushrooms, unidentified problems, regulatory samples, and household molds. About 91% of samples received originated in NC. Insects new to NC included crapemyrtle scale (*Eriococcus lagerstroemiae*) on crape myrtle (*Lagerstroemia indica*), *Aleurocybotus graminiculus* (whitefly; Aleyrodidae) on fountaingrass (*Pennisetum alopecuroides*), and hollyhock thrips (*Pseudophilothrips varicornis*) on Hibiscus. Other notable samples included 1) *Lasioidiplodia* sp. on cotoneaster from a nursery. The symptoms were typical of fire blight, but the fungus was consistently recovered from discolored wood; 2) *Calonectria* sp. identified on *Sarracenia* from two different locations in a 2-wk time period. We are awaiting identification of the species, but it is not *C. morganii* (*Cylindrocladium scoparium*), the only reported

species on this host; 3) *Colletotrichum* sp., possibly *C. sansevieriae*, was recently was identified on *Sansevieria*. If confirmed, this species would new to NC. Other activities included field days, tours, course lectures and labs, and day-long diagnostic workshops, one for 20 CES Agents and one for 13 graduate students. M. Munster and M. Bertone conducted five bi-monthly live webinars in 2016; total attendance was more than 300 County Agents and Master Gardeners.

Evaluation of current fungicides for efficacy against cucurbit downy mildew on cucumber

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Cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is one of the most devastating diseases of cucumber and can lead to complete yield loss. The state of Florida makes up 27% of U.S. production of fresh cucumbers adding \$47 million to the state's economy. Nearly continuous production of cucurbits throughout Florida, puts high pressure on current fungicides labelled for CDM, so resistance is always of concern. On 22 Sep, plots were established at the University of Florida's Gulf Coast Research and Education Center in Balm, FL to assess the efficacy of current fungicides on CDM of cucumber. In total 21 treatments were evaluated during a natural outbreak of *Pseudoperonospora cubensis*. Fungicide treatments were applied weekly from 18 Oct through 14 Nov. Plots were monitored regularly for CDM, and rated on 24 Oct, 3 Nov, 10 Nov, 21 Nov, 2 Dec, and 9 Dec. After the last fungicide application on 14 Nov, ratings on 2 Dec and 9 Dec were performed to assess residual activity of each treatment. AUDPC was calculated for each treatment at the completion of the trial. AUDPC results demonstrated Orondis Opti A, Omega, Ranman, Zampro, and Presidio provided the greatest level of disease control. Residual activity ratings on 9 Dec revealed Orondis Opti A and Omega gave the longest control ranging from 1.5 - 5% disease severity. These results will be applied to future spray program trials for the management of CDM and serve to manage resistance among new chemistries.

Louisiana State University Plant Diagnostic Center Annual Report

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The LSU Plant Diagnostic Center is a member of the Southern Plant Diagnostic Network. The mission of the Diagnostic Center is to provide statewide diagnostics of plant health problems to the residents and Extension personnel of Louisiana. During 2016, a total of 796 routine physical diagnoses, 498 digital diagnoses and 55 site visits were made. Eighty five plant tissue samples and 232 citrus plant tissue samples were processed for Sudden Oak Death Survey and Citrus Clean Plant Network, respectively. Extension outreach program included seven plant disease factsheets, three ornamental and turfgrass certifications programs, nine master gardener sessions, 21 extension talks and a newly revised Home Citrus Production Guide. A national pest alert featuring Boxwood dieback was also developed in 2016. Rose rosette disease and its vector *Phyllocoptes fructiphilus* were confirmed for the first time from Knock Out roses in Louisiana.

The Arkansas Plant Health Clinic

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The Plant Health Clinic program focuses on diagnoses in addition to written information, presentations, and workshops. Outreach efforts through newsletters, displays, and presentations focus on education of Extension personnel, homeowners, growers, and professional nurserymen and Master Gardeners. This program is designed to educate, support, and assist Extension personnel, the citizens of Arkansas, and agriculture through diagnostic services and education on those topics relating to plant diseases. The Plant Health Clinic also supports research in the state with diagnostic services and collaboration. For the reporting period of June 30, 2015 to September 29, 2016, the Plant Health Clinic processed 6,026 samples, and the nematode lab processed 2,622 for a total of 8,658 samples. One hundred thirty-two Plant Health Clinic Newsletter articles were published in forty-four newsletters. Thirty four plant disease presentations were given to 4,030 Master Gardeners and members of the Arkansas Green Industries Association. Eleven thousand two hundred and fifty-eight people were reached through educational displays at Field Days and Flower and Garden shows. The chief diagnostician was an author on "First Report of Citrus leaf blotch virus in Peony in the U.S.A. and the world", J. C. Gress, S. Smith and I. E. Tzanetakis, Dept. of Plant Pathology, University of Arkansas, Fayetteville AR 72701.

A phylogenetic network of the soilborne fungal pathogen *Sclerotium rolfii* in the Southeastern U.S.

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Stem rot is one of the most important soilborne diseases in the Southeastern U.S., causing significant yield loss in tomato, pepper, and peanut. Population structure and genetic variation of the causal agent, *Sclerotium rolfii*, is yet to be explored for Southeastern isolates, despite significant variability in colony morphology and host virulence. Mycelial compatibility groups have indicated the possibility of genetic diversity and previous phylogenetic analysis of the non-coding internal transcribed spacer (ITS) region indicate sequence variation among Florida isolates. The objective of this study was to determine the number of genotypes in the Southeastern U.S. using sequence analysis of a protein coding gene. Thirty-five isolates infecting peanut in Florida, Georgia, and Alabama were collected and whole genomic DNA was extracted. RNA polymerase II subunit I (Rpb1) protein was amplified and sequenced. Sequences were analyzed using the Templeton, Crandall, Sing (TCS) algorithm for determining phylogenetic networks, where each node in the network is a genotype and links between nodes represent mutational steps. This study reports the first phylogenetic network for *S. rolfii* in the

Southeastern U.S. using a protein coding gene sequence. Genotypes in the Southeastern U.S. were distinct from previously characterized Mediterranean genotypes. Knowledge of gene flow among populations of *S. rolfsii* is needed for durable host resistance and can improve disease management.

Impact of reduced fungicide sensitivity in *Fusicladium effusum* on fungicide efficacy under field conditions

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Scab, caused by the fungus *Fusicladium effusum*, can be effectively managed using multiple fungicide applications per season, but resistance to some of these fungicides has severely impacted scab management. Monitoring resistance with in vitro bioassays can be beneficial but is not sufficient to accurately predict performance of a fungicide in an orchard. In 2016, scab epidemics were tracked on replicated pecan trees each receiving ten applications of fenitrothion (TIN; Super Tin 4L), tebuconazole (TEB; Orius 3.6F), azoxystrobin (AZ; Abound), AZ + TEB, TIN + TEB, or no fungicide. Scab incidence and severity were assessed every 14 days and conidia from infected leaf samples were screened for sensitivity to TIN and TEB every 28 days. Leaf scab incidence increased linearly from 25 May to 20 July and increased significantly faster on nontreated trees and trees treated with TIN, TEB, or TIN + TEB than on those treated with AZ or AZ + TEB. Nut scab incidence was significantly lower on trees treated with AZ or AZ + TEB when compared to all other treatments and nut scab severity was significantly lower on trees receiving TIN, AZ, AZ + TEB, or TIN + TEB than on nontreated trees. Previous monitoring has shown reduced sensitivity to TIN and TEB in this orchard. However, critical fungicide sensitivity thresholds to better predict fungicide performance could not be established due to an inconsistent relationship between sensitivity and disease control during the season.

Host-specialized populations of *Corynespora cassiicola* causing emerging target spot epidemics in the southeastern U.S.

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Emerging plant diseases are caused by changes in the pathogen or host populations, or a change in the environment. *Corynespora cassiicola* is a ubiquitous fungus causing recent outbreaks including target spot of cotton, soybean and tomato in the southeastern U.S. This study aims to understand the emergence of the target spot epidemics by developing microsatellite markers for *C. cassiicola* populations to assess genetic variations within each of the identified populations. Also, we want to determine the host range of populations by testing pathogenicity and virulence of *C. cassiicola* populations to cotton, soybean, tomato, and cucumber. Two hundred sixty-five isolates were genotyped with 13 microsatellite markers, and analyzed for genotypic diversity, clonal composition, and population structure by host and geography. Genotypic diversity revealed genetic variation within each of the populations collected from different hosts, with the cotton population dominated by clonal genotypes and showing the least diversity. In addition, *C. cassiicola* populations on different host species were genetically distinct and structured based on host species. Lastly, 40 representative isolates were tested for pathogenicity and virulence on cotton, soybean, tomato, and cucumber cultivars. Isolates were most aggressive when inoculated onto the same host of origin. These results suggest that the cause of the emerging epidemics in the southeastern U.S. is likely due to an introduction of host specialized isolates or the evolution of more aggressive strains on each host. Knowledge on the pathogen population diversity is critical for screening for cultivar resistance and in determining appropriate fungicides for target spot management.

Characterization of Tvhyd1, a *Trichoderma virens* hydrophobin

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Trichoderma virens is a filamentous fungus known for its beneficial properties when associated with plant roots. The mechanisms used by the fungus to detect roots, initiate colonization, and induce systemic resistance are not well defined. We hypothesize that hydrophobins play a critical role in plant-fungal interactions. Hydrophobins are small proteins containing a conserved pattern of eight cysteine residues and are classified into two groups based on hydrophobicity. We have identified a putative class I hydrophobin (Tvhyd1) in *T. virens* based on gene expression profiles recorded with microarray data sets in presence of maize and tomato, genomic analysis and protein modeling, and generation of a hydropathy plot. In the presence of roots in a hydroponic system, gene expression occurred between 30 and 54 hours post inoculation. Additionally, there was no expression at 48 hours in PDB or minimal medium. Gene knockout mutants were generated and confirmed by PCR and Southern hybridization and subjected to phenotypic assays. There were no significant differences between the wild type and the mutants for sporulation, attachment to hydrophobic substrates, biocontrol against *Rhizoctonia solani*, or growth rates on agar. However, a significant decrease in root colonization ability in maize was detected by qPCR analysis after 3 days of co-cultivation. The mutants also showed growth inhibition in confrontation with *Pythium ultimum* after 6 days of growth on agar.

***Pseudomonas floridae* sp. nov., a novel bacterial pathogen isolated from tomato**

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An unusual fluorescent pseudomonad was isolated from tomato exhibiting symptoms similar to bacterial speck. Strains were fluorescent, oxidase and arginine negative, gave an HR on tobacco and produced a soft rot on potato. However, the strains produced unusual yellow, mucoid growth on hypersucrose medium not typical of levan. Based on multilocus sequence analysis using 16s rRNA, *gap1*, *gltA*, *gyrB* and *rpoD*, these strains formed a distinct phylogenetic group in the genus *Pseudomonas*, and were most closely related to *Pseudomonas viridiflava* within the *P. syringae* complex. Whole genome comparisons, using average nucleotide identity based on blast (ANIb), of strain GEV388 of the unusual pseudomonads and representative strains from different genomospecies of *P. syringae* complex revealed that phylogroup 7 *P. viridiflava* strain UASW0038 and the *P. viridiflava* type strain ICMP2848 were the closest relatives with 86.59% and 86.56% ANIb, respectively. In silico DNA-DNA hybridization using the genome-to-genome distance method gave an estimate of 31.1% homology between GEV388 and *P. viridiflava* ICMP2848^T, suggesting the strains are different species. These results together with Biolog GEN III, fatty acid methyl ester (FAME) and phylogenetic analysis, demonstrate that this group represents a novel species member of the genus *Pseudomonas*. The name *Pseudomonas floridae* sp. nov. is proposed with GEV388^T as the type strain.

Oxylipins control *Trichoderma virens*-mediated Induced Systemic Resistance in Maize

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Colonization of roots by *Trichoderma virens* provides plants with benefits that include enhanced growth and induced systemic resistance (ISR), a robust priming of systemic defenses against a broad range of pathogens. One initial component of defense priming is the production and secretion of the protein SM1 by *T. virens*. *T. virens*-mediated ISR in maize acts through the suppression of the 9-lipoxygenase *LOX3*, suggesting that oxylipins play a role in regulating ISR. Transcriptomic analysis of *lox3* mutant roots revealed overexpression of a 13-lipoxygenase *LOX10*, a gene responsible for production of green leaf volatiles (GLVs) and traumatin. Remarkably, compared to control plants, the *lox10* mutant colonized by *T. virens* displayed increased susceptibility to, the anthracnose leaf blight pathogen, *Colletotrichum graminicola*. We hypothesize that both *LOX3* and *LOX10* coordinate the production of root-derived long-distance signals that govern *T. virens*-mediated ISR. To test this hypothesis, xylem-sap from *T. virens*-treated donor plants were transfusion into untreated receiver plants. While transfusion of xylem-derived sap from *lox3* or *T. virens*-colonized WT maize confers systemic resistance to receiver plants, transfusion with xylem-derived sap from *T. virens*-colonized *lox10* increased susceptibility in receiver plants. These findings provide genetic evidence that the long-distance ISR signals originate in the roots and travel through the xylem to confer systemic resistance.

Assessing applications of secondary nutrients as a management strategy for charcoal rot of soybean

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Charcoal rot (CR) of soybean, caused by *Macrophomina phaseolina* (Tassi) Goid (*Mp*) a ubiquitous soilborne fungus, causes significant yield losses. CR is observed in years characterized by high temperatures and limited rainfall, but can also occur in poorly irrigated fields since the disease is incited by stress. Although infection can be initiated during seedling stages, most symptoms are not observed until reproductive development. However, with a general lack of resistance available within commercially available germplasm the management options for CR are extremely limited. The objective of this research was to reduce charcoal rot plant tissue colonization by supplementing with 1,120 kg/ha of calcium (Ca) and magnesium (Mg), alone and in combination, at three different application timings (pre-plant, at-plant, pre-plant fb at-plant). Between 2014 and 2016 non-irrigated, *Mp*-inoculated field trials were conducted with a CR-susceptible and -moderately-resistant cultivar. Non-inoculated, non-treated plots served as controls. Disease severity was visually assessed in root tissue. *Mp* colonization was quantified from ground root tissue and enumerating colony forming units (cfus) in a *Mp* semi-selective medium. An approximate 10 to 20% reduction in disease severity and colonization was observed with some treatments. Although, a steady increase in cfus occurred throughout the season regardless of treatment and cultivar, yield was not negatively impacted.

Performance of root-knot nematode resistant cotton varieties in fields infested with *Rotylenchulus reniformis*

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The Southern root-knot nematode (*Meloidogyne incognita*) and the Reniform nematode (*Rotylenchulus reniformis*) are economically important pests of upland cotton (*Gossypium hirsutum*) throughout Texas. Currently, chemical options for managing nematodes are limited. Cotton varieties possessing partial resistance to *M. incognita* were developed in the 1970's; however, yield and quality of these varieties were inherently low. More recently, varieties with improved resistance to *M. incognita* have been released with many varieties having a dual gene system. Sources resistant to *R. reniformis* are available in *G. hirsutum*, but have low yield potential. Trials evaluating the performance of varieties resistant to *M. incognita* were conducted from 2014-2014. Lint yields were similar among varieties under low *R. reniformis* pressure. Increases of 15 to 39% were observed the dual gene resistant varieties Phytogen 417WRF or Phytogen 427WRF over the susceptible Phytogen 499WRF. Such trends were not observed with other dual gene systems, such as Deltapine 1454NR B2RF or Deltapine 1558NR B2RF or single gene systems such as Phytogen 367WRF, Stoneville 4946GLB2, or Fibermax 2011GT. Preliminary data suggests that *R. reniformis* reproduction was lower for the two varieties possessing the dual gene resistance.

Additional studies are needed to better quantify reproduction of *R. reniformis* on these and other varieties possessing resistance to *M. incognita*.

Characterization of functional subnetwork modules associated with ear rot and fumonisin biosynthesis in maize pathogen *Fusarium verticillioides*

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Fusarium verticillioides is a fungal pathogen causing ear rot and fumonisin contamination in maize, posing significant food safety and security risks. Improving our knowledge of cellular and genetic mechanisms associated with pathogenesis and toxin biosynthesis facilitate the development of innovative control strategies. In this study, we performed a systematic network-based analysis of large-scale *F. verticillioides* RNA-seq datasets to identify potential genetic modules that are involved with virulence and fumonisin regulation. Specifically, our key aims were to identify subnetwork modules in *F. verticillioides* co-expression network that are significantly differentially expressed in two different maize lines (moderately resistant vs susceptible), then to characterize predicted hub genes in these functional subnetwork modules. We identified one putative hub gene in an independent subnetwork module. This gene encodes Frn1, a hypothetical protein devoid of any known domain. We hypothesized that this gene operates collectively with other linked genes in the subnetwork to regulate fumonisin biosynthesis and pathogenesis. Our data demonstrated that *FRN1* has little impact on vegetative growth and fertility but plays an essential role in fumonisin production and stress response. *FRN1* showed polymorphism in the different field isolates in Texas. Protein alignment study revealed high levels of similarity within *Fusarium* species, while predominantly absent in other fungal species.

HAP3 of *Phomopsis longicolla* influences growth, development, and pathogenesis

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Phomopsis longicolla (Hobbs), the most important soybean seed pathogen, is the causal agent of *Phomopsis* seed decay. Currently, little is known about the molecular basis of pathogenesis in *P. longicolla*, in part because crucial tools for molecular genetics, such as targeted gene deletion, have not been demonstrated in this organism. In other filamentous fungi, the heterotrimeric CCAAT-binding complex is involved in diverse aspects of growth and development, and pathogenesis. In this study, a putative component of the CCAAT-binding complex (HAP3) was identified in *P. longicolla* and characterized through functional genomics. *HAP3* gene was successfully deleted via homologous recombination, and the mutant was genetically complemented via reintroduction of the wild-type gene. Deletion of *HAP3* substantially impaired growth, development and the ability of *P. longicolla* to colonize soybean seeds and stems. A $\Delta hap3$ strain (H101) also presented massive transcription reprogramming while colonizing soybean seeds. A total of 2080 upregulated and 1732 downregulated genes were found in H101, accounting respectively for 12 and 14% of *P. longicolla* predicted protein coding genes (adjusted $P < 0.05$). This study reviews the important role of the *HAP3* in growth, development and pathogenesis of *P. longicolla* and illustrates the feasibility of targeted gene deletion in *P. longicolla*.