# 2012 Caribbean Division Meeting Abstracts

Abstracts presented at the APS Caribbean Division meeting in South Padre Island, Texas, April 16–18, 2012. 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: Ananthakrishnan, G., Hu, H., and Brlansky, R. H. 2012. Development and applications of primers and probe for genus specific detection of '*Candidatus* Liberibacter species' by real-time PCR. (Abstr.) Phytopathology 102(Suppl. 6):S6.1. http://dx.doi.org/10.1094/PHYTO-102-11-S6.1.

**Development and applications of primers and probe for genus specific detection of '*Candidatus* Liberibacter species' by real-time PCR**

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Phytopathology 102(Suppl. 6):S6.1

Citrus huanglongbing (HLB, also called greening), is currently one of the most devastating citrus diseases in the world. Three types of disease are reported, and they are associated with three different '*Candidatus* Liberibacter species', which belong to the alpha subdivision of Proteobacteria. The most widely used diagnostic assays for HLB are conventional and real-time PCR (qPCR) using primers/probes based on *16S* ribosomal RNA (rRNA) genes, however, some HLB samples are undetectable by them (*i.e.* false negative) due to unknown reasons. We reported here the development and applications of a set of genus specific primers/probe based on *rpoB* gene (encoding the beta-subunit of RNA polymerase) for liberibacters detection with qPCR. This genus specific primers/probe set were validated in both SYBR Green I and TaqMan formats. When applied to different samples (including samples from different plant species like citrus, citrus relatives, tomato, potato, carrot; and samples from different geographic areas worldwide), it is proved useful to detect the three reported HLB bacteria ('*Ca*. L. asiaticus', '*Ca*. L. africanus', and '*Ca*. L. americanus') and '*Ca*. L. solanacearum' associated with the potato Zebra Chip disease. This generic primer/probe offered a new option for HLB diagnosis, thus should help in HLB management.

**Functional characterization of SDA1, a putative transcriptional activator of the polyol pathway, in *Fusarium verticillioides***

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Carbon metabolism in fungi provides not only metabolic energy, but also intermediary metabolites for a variety of cellular needs. In general, glucose, one of many 6-carbon sugars, is primarily absorbed for glycolysis. Significantly, carbon metabolism in fungi is far more complex than our current understanding. The polyol pathway is an alternative mechanism where the unused glucose in cells is reduced to sorbitol by aldose reductase, and subsequently oxidized to fructose by sorbitol dehydrogenase. In humans, deficiency in this pathway has been correlated with microvascular complications and diabetes. In *Fusarium verticillioides*, we identified a gene encoding a putative $Cysteine_2$ $Histidine_2$ transcription factor, and hypothesized that it is involved in the activation of polyol pathway. We designated this gene *SDA1* (sorbitol dehydrogenase activator). *SDA1* null mutation showed complete inhibition of growth when using sorbitol as the sole carbon source. In addition, the growth of null-mutant strain was severely impaired in the presence of other polyols, such as mannitol and glycerol. However, *SDA1* null mutation had an unexpected effect on fumonisin B1 (FB1) biosynthesis; we observed three times higher level of FB1 when compared to the wild-type progenitor. Significantly, *F. verticillioides* Sda1 protein shares 65% identity, and 76% similarity with *Trichoderma reesei* Ace1. We are complementing *F. verticillioides SDA1* null mutant with *T. reesei Ace1* to test whether they are functional orthologs. Our data suggest that Sda1 is a transcriptional activator necessary for the utilization of polyols in *F. verticillioides*. Further study is needed to determine how Sda1 regulates genes associated polyol metabolism in fungi.

**Identification of bacteria used to increase larval survivorship of fruit flies used in sterile insect technique**

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The Mexican fruit fly, *Anastrepha ludens* (Loew) is a major pest that attacks the fruit groves of Texas, Southern California and Arizona. Female Mexican fruit flies can lay up to 1500 eggs during their lifetime representing a major threat to citrus and other fruit industries. One method to control the population of Mexican fruit flies is through biocontrol, specifically sterile insect technique (SIT). Key to this method is raising millions of sterile males per week for release. Because of the sheer number of sterile males released each week, the reproductive success of female fruit flies is severely reduced. For SIT to be successful, sterile males must be released constantly and bacteria are now being considered as a resource to decrease the cost of SIT by increasing survivorship and mating success of the sterile males. Bacteria can play a large role in the success of a fruit fly colony as a probiotics or failure as pathogens that, if introduced, can wipe out a colony. To assess the potential of a bacterium as a probiotic or pathogen, the identity of the bacterium must be known. Bacteria were isolated from the eggs and larvae of A. ludens. The universal bacterial primers were used to amplify and sequence the 16s rDNA region. The sequences were aligned against the GenBank nucleotide database using the megaBLASTn algorithm. Of the 115 bacterial sequences analyzed, only seven were not identified using BLASTn or BLASTx. The identified bacteria included representatives from the phyla Proteobacteria (98), Bacilli (3), Actinobacteria (5) and Bacteroidetes (2). The gamma subdivision of Proteobacteria included 90 of the identified isolates with the vast majority, 60 isolates, belonging to Pseudomonadeles. The analysis found 13 strains of *Pseudomonas aeruginosa*, a known pathogen of the fruit flies. Additionally, the two Bacteroidetes are similar to probiotics used for humans.

**Novel seed treatment methods using non-thermal plasma for control of seedborne pathogens in rice**

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One of the most common entry points for pathogens into a crop system is via seed. Oftentimes, domestically and internationally traded seeds introduce new pathogens into previously uninfected crop fields. To mitigate this problem, fungicides, hot water or chlorine treatments are used for treating seeds before planting. However, these treatments are labor intensive, dependent on environmental conditions and hazardous to humans and animals. Thus, novel alternative methods are necessary to facilitate the production and distribution of pathogen-free seed. Plasma technology offers the possibility of an effective new seed treatment method. Non-thermal plasma is composed of partially ionized, chemically reactive gases and possesses antimicrobial properties. In this work, a non-thermal plasma seed treatment was tested for two common seedborne pathogens in rice: *Gibberella fujikuroi* (causal agent of bakanae disease) and *Burkholderia glumae* (causal agent of panicle blight). Non-thermal plasma reduced the number of colony forming units by almost 100% after 30 seconds of the plasma seed treatment. To evaluate the safety of the plasma treatment on seeds, rice seeds were exposed to plasma for 3 and 20 minutes and incubated in moisturized petri dishes at room temperature. The seeds' germination and growth rates were measured and recorded over a span of two weeks. It was found that the germination and growth rates of seeds treated with plasma for 3 and 20 minutes were similar to those of the untreated control seed. Morphologically, seedlings from the treated seeds were similar to those of the control. In conclusion, non-thermal plasma is an effective alternative seed treatment method possessing antimicrobial activity against both bacterial and fungal pathogens without any harmful effect on seed germination and vigor.

**The role of nutritional supplements in managing HLB**

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Citrus varieties infected with the bacterium, Candidatus Liberibacter asiaticus, the causative agent of the citrus greening disease (Huanglongbing; HLB), exhibit specific visual symptoms synonymous with nutritional deficiencies. The visual symptoms of HLB-infected trees in Florida vary with the season as does the presence of HLB in different organs. Young fruit symptoms include misshaped fruit, similar to those seen for calcium deficiency and aborted seeds analogous to boron deficiency. Mature fruit symptoms include soft fruit, similar to potassium deficiency, leaky vesicles and early abscission, as seen for calcium and boron deficiencies. Leaf symptoms include vein corking and enlargement, comparable to boron deficiency, blotchy mottle, resembling zinc and manganese deficiencies. Late season twig dieback in HLB-infected trees in Florida is a prevalent symptom, which is akin to zinc and manganese deficiencies. Because of the numerous similarities between HLB-infected and nutritional deficiency symptoms in Florida, nutritional protocols designed to provide specific nutrients required by the plant during the different stages of development have been adopted by the industry to mitigate/mask HLB symptoms. None of the nutritional protocols currently in use in Florida have cured HLB infections in the trees; however, they appear to have provided stabilization of fruit production. This presentation will attempt to provide information regarding nutritional protocols and yield data of KeyPlex commercial trials conducted for the past four years in HLB-infected Florida citrus groves.

**Transmission of phytoplasmas associated with Texas Phoenix palm decline (16SrIV-D) to Pritchardia pacifica by the planthopper Haplaxius crudus (Cixiidae) in Yucatan, Mexico**

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In the 1970's and 1980's "coconut lethal yellowing" (LY) was known as a single phytoplasma disease in Jamaica and Florida. The premature dropping of the nuts regardless of size, the blackening of new inflorescences and ascending yellowing of the leaves were considered as reliable diagnosis of LY. At the same time, in Florida, other palm species like *Phoenix* spp. *Pritchardia* spp. or *Veitchia merrilii* were affected by wilts showing similar symptoms. As phytoplasmas were diagnosed by electron microscopy in these species, they were also claimed to be affected by LY. Field evidence favored the planthopper *Haplaxius (Myndus) crudus* (Cixiidae) as a potential vector. Several trials to transmit LY by this insect started in Jamaica and Florida in the 1970s. After 10 years of research, transmission of LY disease to coconut (*Cocos nucifera*) was obtained in Florida, but not in Jamaica. In Tabasco State (Mexico) research conducted between 2006 and 2010 failed to obtain LY transmission by *M. crudus* despite 70,000 field collected insects placed on coconut palms in cages. Trials performed in 2011 in Yucatan showed that H. crudus collected in the field on various palm species (*Thrinax radiata*, Sabal spp., *Washingtonia* spp., coconut) were able to transmit a disease to *Pritchardia pacifica* but not to coconut palm. Phytoplasmas associated with the symptoms in *Pritchardia* were of the group 16SrIV-D, known as "Texas Phoenix palm decline". In fact it has recently been shown by PCR and sequencing that at least five subgroups of phytoplasmas, 16SrIV-A to F, occur in different palm species in the Caribbean area including west central Florida. The question as to which insect is associated which 16SrIV phytoplasma remains open. New trials are in progress.

**Occurrence of citrus viroid III in the northeast of Mexico**

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We analyzed the presence of citrus viroid III (CVd-III) in 40 samples of 18 varieties of citrus from Marin, Nuevo Leon and Hidalgo, Tamaulipas using molecular techniques. We used tree bark and leaves, which were sampled randomly, and then RNA was isolated using Trizol reagent. We used one-step RT-PCR with two specific primers pairs; and the amplified products were separated by electrophoresis and visualized in UV light, sequenced and compared in the NCBI GeneBank. By 19 samples of citrus from Nuevo Leon state, 8 were positive to CVd-III; and from 21 samples of Tamaulipas, 11 were positive represented the 42% and 52% respectively of the total trees analyzed. The sequences of the amplified products showed 97–99% of homology compared with an isolate from Cuba (AJ630358.1). Citrus species and varieties with CVd-III were: mandarin, Carrizo citrange and Valencia orange. The presence of citrus viroid III in groves of Nuevo León and Tamaulipas can be attributed to propagation by grafting and that this viroid is not regulated and it is not part of the certification programs of citrus plants in Mexico. To our knowledge this is the first report of the presence of CVd-III in the state of Tamaulipas.

**In vitro screening of plant materials as biofumigants for the management of *Rhizoctonia solani* in rice**

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Sheath blight caused by *Rhizoctonia solani* AG 1-1A is one of the major rice diseases in the southern rice production areas in the United States. No commercial rice cultivars with complete resistance to sheath blight are available. Cultural management options including fallow and crop rotation can be used but not always applicable for most rice farmers. Therefore, there is a great need to develop environmentally-friendly and sustainable management options. In this study, 11 plant cultivars were evaluated as biofumigants for their inhibition effect on *R. solani*. Tested plants comprised of six *Brassica juncea* cultivars (Brand 199, Ruby Streak, Florida Broadleaf, Green wave, Red giant and Sheali Hong), two Brassica rapa cultivars (Southern Green and Napa), and one cultivar of *Brassica oleracea*, *Eruca vesicaria* and *Crotalaria juncea*. Macerated tissue sample (3 g) of each plant was confined to the lid of a petri dish without physical contact with the 8-mm in diameter disc of *R. solani* on potato dextrose agar (PDA) on the bottom of the petri dish. In addition, the same batch of tissue samples was prepared and mixed at a 1:1 ratio with each of natural soils collected from Texas, Arkansas and Mississippi rice fields. The mixture was exposed to *R. solani* as the aforementioned method. After 48 h of incubation at 25°C, mycelial growth of *R. solani* on PDA plates was measured. All plants evaluated had some level of growth inhibition on *R. solani*. Four *B. juncea* cultivars (Brand 199, Ruby Streak, Florida Broadleaf and Green wave) consistently provided the greatest inhibition in all the soil types. *C. juncea* and *B. rapa* "Napa" had the least *R. solani* inhibition in all the soils evaluated. Results of this study indicate that biofumigant plants may provide an alternative disease management option for sheath blight of rice.

**Sporulation and field movement of atoxigenic strains of *Aspergillus flavus* used for the management of aflatoxin in corn and cotton**

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Aflatoxin contamination accounts for annual losses of $32 million of corn and cotton in Texas. Two atoxigenic strains of *Aspergillus flavus*, AF36 and Afla-Guard, are labeled for its management. The purpose of this study was to measure differences in the ability of these strains to sporulate and to track movement of their conidia in corn and cotton fields. Sporulation was evaluated by incubating the two strains on their commercial formulations (inoculated on cereal grains) at six constant humidity levels ranging from 0–100%, using closed chambers with saturated salt solutions. Conidial production by Afla-Guard was 3-fold greater than that of AF36 at 100% humidity.

Sporulation of the two strains was also evaluated on one substrate by inoculating their conidia on sterile, hulled barley. After 3 days, there was a 234-fold increase in conidia recovered from the barley inoculated with Afla-guard, compared with a 21-fold increase in conidia recovered from the AF36-inoculated barley. To measure conidial movement, an Afla-Guard nitrate non-utilizing mutant, colonizing autoclaved corn seed, was placed at one point in a field. For detection, aliquots, washed from leaf samples, were plated onto a medium containing potassium chlorate. The mutant was recovered at a maximum distance of 6.4 m in corn fields along the same row and as far as 10.2 m across rows from the point source. In cotton fields, the mutant was recovered at 9.1 meters along the same row and 6.1 m across rows from the point source. There was no recovery at 24.3 m from the point source, the maximum distance evaluated. These data suggest that the Afla-Guard strain sporulates better than the AF36 strain, which may be a factor in effectiveness for biological control. Additionally, the movement data suggest that plots in field trials may not need wide separation in order to avoid cross contamination.

**Selective citrus relatives as alternative hosts of citrus huanglongbing in Florida**

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Citrus huanglongbing (HLB) is a devastating citrus disease. HLB in Florida is associated with bacterial pathogen Candidatus Liberibacter asiaticus and mainly transmitted by Asian citrus psyllid (*Diaphorina citri*). With the rapid spreading of HLB in Florida, citrus relatives required study due to their potential as alternative hosts. Some citrus relatives previously have been listed as hosts of the psyllid and/or the associated bacterium based on field surveys or PCR tests on field samples, but their status has never been systemically studied. In this work, several citrus relatives, such as *Severinia buxifolia*, *Citropsis gilletiana*, *Esenbeckia runyonii*, *Zanthoxylum fagara*, *Amyris texana*, and *Choisya* spp., were studied in the greenhouse to investigate their alternative host status. All possible transmission pathways for each plant were tested with 3 repeats of psyllid transmission experiments as well as grafting where compatible. After inoculation, plants were monitored for symptom development and tested by real-time PCR. The results showed that all plants studied were able to be infected by *Ca*. L. asiaticus, but only some (e.g. *S. buxifolia*) could serve as alternative transmission hosts due to various reasons. Our work has provided experimental evidence for the alternative host status of numerous selective citrus relatives, which is helpful in HLB management in Florida.

**Molecular analysis of the West Indian fruit fly *Anastrepha obliqua* collected in Jamaica using mitochondrial markers**

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The West Indian fruit fly, *Anastrepha obliqua* Macquart, is a major pest of mangos and plums. Its effects on the production of some fruit are so great that the profitability of growing the fruit is not possible. The fly is widely distributed across subtropical and tropical regions of the New World which includes Mexico, Central and South America, as well as the Caribbean. *A. obliqua* is currently placed in the *A. fracterculus* species complex, a large group of New World Tephritidae. This group has been very difficult to resolve because of limited informative morphological traits creating multiple cryptic species. One result of not being able to accurately identify the pest is that the host range has been difficult to ascertain. Also, from a management perspective, detecting pathways of introduction is limited when identification diagnostics are inadequate. Although ecological and molecular studies have been performed on *A. obliqua*, the taxonomic status of different populations of *A. obliqua* has not been determined. By studying particular populations with molecular markers, resolution of the species complex is possible. The population examined in this study represents *A. obliqua* from Jamaica. One hundred seven samples were collected and DNA was isolated. Molecular barcoding using COI DNA sequences were used and analyzed for mitochondrial haplotypes. When compared to other populations of *A. obliqua*, the Jamaican population has four haplotypes of which three haplotypes have not been identified in any other population. This information will be used for understanding the current distribution of this species and will be of value for pest management strategies.

**Microsatellite based genetic diversity and structure of '*Candidatus* Liberibacter asiaticus' associated with citrus Huanglongbing worldwide**

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Huanglongbing (HLB) is one of the most destructive citrus diseases in the world. This disease is associated with the presence of a fastidious, phloem-limited α-proteobacterium, '*Candidatus* Liberibacter asiaticus', '*Ca*. Liberibacter africanus' or '*Ca*. Liberibacter americanus'. HLB-associated Liberibacters have spread to North America and South America in recent years. While the causal agents of HLB have been putatively identified, information regarding the worldwide population structure and epidemiological relationships for '*Ca*. L. asiaticus' is limited. In this study a panel of seven polymorphic microsatellite markers was developed from '*Ca*. L. asiaticus', and used for conducting genetic analysis of this bacterium from a worldwide collection. Two hundred eighty seven isolates from U.S.A. (Florida), Brazil, China, India, Cambodia, Vietnam, Taiwan, Thailand, and Japan were analyzed. Results showed that the genetic diversity of '*Ca*. L. asiaticus' is higher in Asia than Americas. Clustering analyses from UPGMA method and STRUCTURE program consistently identified three major genetic groups worldwide. Isolates from India were genetically distinct. East-southeast Asian and Brazilian isolates were generally included in the same group; a few members of this group were found in Florida, but the majority of the isolates from Florida were clustered separately. eBURST program predicted three founder haplotypes, which may have given rise to three groups worldwide. A similar genetic makeup of 'Ca. L. asiaticus' isolates between Brazil and east-southeast Asian dominant group suggests the possibility of a common origin. While the sources of the dominant 'Ca. L. asiaticus' in Florida were not clearly understood, the introduction of less-pervasive groups may have been introduced directly from Asia or via Brazil. Notably, the recent outbreak of HLB in Florida probably occurred through multiple introductions.

**Use of scanning electron microscopy in the speciation of *Gliocephalotrichum* spp. in rambutan (*Nephelium lappaceum* L.)**

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Rambutan is a tropical tree fruit crop native to Malaysia. Worldwide, fruit rot is a limiting factor for fruit quality. In 2011, fruit rot was observed on rambutan at the USDA-ARS Tropical Agricultural Station in Mayaguez, Puerto Rico, and was attributed to *Gliocephalotrichum* spp. Light microscopy (LM) and molecular characterization are tools to differentiate *Gliocephalotrichum* spp. The use of scanning electron microscopy (SEM) has been a successful tool to differentiate powdery mildews at species level. To test the potential of SEM to speciate *Gliocephalotrichum* spp., two pathogenic species, *G. bulbilium* (*Gb*) and *G. simplex* (*Gs*), and a saprophytic *G. bacillisporum* (*Gba*), were grown on rambutan spintem (fruit hair-like appendage) tissue (*Gb* and *Gs*) and onto carnation leaf agar (*Gba*). Plant tissue was prefixed in 3% gluteraldehyde and 0.1 M potassium phosphate buffer, (pH = 7.2) for 24 h at 8°C. Samples were dehydrated with ethanol, followed by critical point drying. Detailed SEM observations of stipes under penicilli, basal conidiophore cells, conidiophore stalks, and patterns of the surfaces of conidia and chlamydospores were obtained. From the base to mid-section of the stalk, verrucose-like patterns were observed on *Gb*, but smooth patterns on *Gba* and *Gs*. Conidial surface of *Gb* were ornamented with undulated fibrillar patterns, whereas conidia of *Gba* and *Gs* were smooth. Conidia of *Gs* were slightly curved at one end but conidia of *Gb* and *Gba* were straight. Chlamydospores of *Gs* were solid and smooth but bulbilloid aggregates of *Gb* were porous. Emergence of the conidiophores and mycelia on plant tissue suggest differences in infection processes among species. Conidiophores of *Gb* and *Gba* emerge through the stomata but conidiophores of *Gs* ruptured epidermal spintems cells. SEM has the potential to be used as a characterization tool for species of *Gliocephalotrichum* on rambutan and other crops.

**The functional role of gliotoxin in the biocontrol agent *Trichoderma virens***

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Fungi of the genus *Trichoderma* are filamentous ascomycetes recognized as biocontrol agents, particularly against phytopathogenic fungi. Mechanisms of biocontrol employed by *Trichoderma* include mycoparasitism and antibiotic production, induction of defense responses in plants, rhizosphere competition, and metabolism of germination stimulants (Howell, 2003). Many species of *Trichoderma* secrete secondary metabolites with antimicrobial activity (e.g. gliotoxin, peptaibols, gliovirin), which may affect the ability of plant pathogens to initiate disease. Gliotoxin is an epipolythiodioxopiperazine toxin (ETP toxin) produced by several different fungi, most notably *T. virens* and

*Aspergillus fumigatus*. Although the role of gliotoxin in *A. fumigatus* as a virulence factor in aspergillosis has been confirmed (Sugui et al., 2007), the function of gliotoxin in the ecology *T. virens* has not be fully defined. The sequenced genome of T. virens contains a cluster of twelve genes that code for the biosynthesis of gliotoxin. In the present study, the non-ribosomal peptide synthetase gene of the cluster, gliP, was disrupted with either a vector containing the hygromycin resistance gene or one containing arg2 as the selectable marker. Three hygromycin tranformants (ΔgliP6, ΔgliP13, and ΔgliP14) and three arginine transformants (ΔgliP44-3, ΔgliP44-4, and ΔgliP44-11) with null expression of gliP and incapable of producing gliotoxin were obtained. These mutants were used to characterize the role of gliotoxin, both physiologically and chemically, in *T. virens*. ΔgliP mutants exhibited phenotypes comparable to that of the wild type with regards to root colonization of maize, biocontrol of *Rhizoctonia solani* and *Pythium ultimum* on cotton, and coiling around the hyphae of *R. solani*. ΔgliP mutants however did grow faster radially on agar plates and were compromised in their ability to degrade the sclerotia of *Sclerotinia sclerotiorum* indicating that gliotoxin may play a role in the mycoparasitism of this fungus.

**Prevalent citrus diseases in Puerto Rico**

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Citrus diseases have destroyed millions of hectares in the world. There are diseases affecting citrus in Puerto Rico, that are causing losses without being detected or identified. A survey was conducted from February 2011 to February 2012 in the major citrus producing areas of the island for detection of citrus greening (CG) and other diseases. Fifteen nurseries and seven commercial orchards located in 20 municipalities were sampled. Symptomatic plants were collected and processed at the Plant Disease Clinic (SPDN) in Juana Díaz. Diagnosis of fungal diseases was carried out by isolation of disease tissue in artificial media, ELISA test for Citrus Tristeza Virus and DAS ELISA for Citrus Variegated Chlorosis. Citrus greening was identified using Polymerase Chain Reaction (PCR) with specific primers (OI1, OI2c) and *Colletotrichum* sp. with primers CgInt, Caint and ITS4. A total of 330 samples were processed and the major diseases identified were anthracnose caused by *C. gloeosporiodes*, fruit spot by *Alternaria* spp., *Candidatus* Liberibacter asiaticus was detected in 6% of the samples in the municipalities of Adjuntas, Cabo Rojo, Coamo, Corozal, Dorado, Isabela, Juana Díaz, Lares, Las Marías, Mayagüez, Ponce, San Sebastián, and Santa Isabel. Citrus Tristeza Virus was positive in 14% of the samples. The introduction of propagative material and fruit imports from other countries represents a threat for exotic diseases entering inadvertently to Puerto Rico. Regular screening in commercial orchards of pathogens of quarantine importance, such as black spot and citrus canker, will be implemented to protect citrus production in the island.

**Use of a toothpick baiting method to detect and monitor *Rhizoctonia solani* that causes large patch in zoysiagrass in southeast Texas**

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Zoysiagrass is among the most economically important warm-season turfgrasses produced and managed by the green industry in Texas. This turfgrass is well-suited to residential and commercial use due to its aesthetic appeal, excellent heat and drought tolerance, and good shade and cold tolerance. Large patch caused by *Rhizoctonia solani* is a serious disease that occurs in the spring and the fall when zoysiagrass is exiting or entering dormancy. The disease weakens turfgrass and reduces its quality, and kills it in a severe infection. Control of large patch is of concern not only to homeowners, but also to sod producers and turfgrass managers in Texas, who encounter significant economic loss as a result of chemical inputs, lower market value and failure to harvest blighted sod. Despite the use of fungicides, control of large patch is difficult due to the biology of the causal agent. Once established in the soil, *R. solani* cannot be eliminated. Disease symptoms usually disappear and turfgrass recovers in the spring as the soil temperature increases. However, disease outbreaks tend to return and patches expand in the same location in the fall when environmental conditions are more favorable for disease development. Before efficient and effective management practices for large patch are developed, the causal agent needs to be properly identified, characterized and monitored. A toothpick baiting method has been developed to effectively isolate *R. solani* and to monitor pathogen activity year-round in zoysiagrass in southeast Texas. Since knowledge is lacking concerning environmental conditions favorable for large patch development, soil moisture was monitored and its relationship with pathogen activity was determined.

**The genetic diversity of *Fusarium verticillioides* in Texas**

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*Fusarium verticillioides* is a ubiquitous fungal pathogen causing ear rot and stalk rot in corn. Notably, the fungus produces fumonisin B1 (FB1), a mycotoxins, on contaminated ears and kernels. FB1 has been shown to cause equine leucoencephalomacia and porcine pulmonary edema as well as being linked to various illnesses in humans. Currently, there is a limited understanding of the distribution of toxic *F. verticillioides* in corn producing areas, and Texas is no exception. The lack of such information hampers applying best management practices to reduce the risk of diseases as well as FB1 contamination. Thus, our research aim was to investigate the population and genetic diversity of *Fusarium verticillioides* in the state of Texas. Samples from every corn-producing county were provided by the Texas State Chemist Office. Fusaria were isolated from these samples through spore serial dilutions and cultured. Once pure isolates were obtained, DNA was extracted and tested for the presence of the *FUM1* gene, a key fumonisin biosynthesis gene, and for mating type genes using PCR. The results were analyzed to correlate *F. verticillioides* mating types and FB1 production to Texas corn-growing counties. We are also performing Random Amplified Polymorphic DNA (RAPD) tests with UBC, OPA, and OPB primers to profile the genetic diversity of *F. verticillioides* isolates in the state. Data generated will be used to create a map reflecting the *F. verticillioides* diversity in Texas.

**Downy mildew on impatiens in Florida: Update on management options for the nursery and landscape**

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Downy mildew has moved into Florida and is currently devastating impatiens in the landscape. The Florida Extension Plant Diagnostic Clinic located at the University of Florida's Tropical Research and Education Center, Homestead, FL received its first sample in early January submitted from a landscape in Palm Beach County. Over the next week, samples started coming in from all over Palm Beach County, FL where it seemed impatiens were dying everywhere. As time progressed, diagnostic samples began to arrive from landscapes further south in Fort Lauderdale, Doral, Miami, and eventually showed up in a large planting of impatiens in Homestead, FL. It's been a very mild winter in South Florida with recent daytime temperatures reaching the upper 80's, but the cooler nighttime temperatures (50's to70's) combined with high relative humidity (average 85%) created the perfect environment for disease. Details on the history of the pathogen *Plasmopara obducens* and where it's been reported throughout the US, its impact on impatiens in nurseries and landscapes throughout Florida, and results from management trials will be presented.

**'*Candidatus* Liberibacter solanacearum' translocation and quantification in potato and tomato**

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'*Candidatus* Liberibacter solanacearum,' (Lso) is the causative agent of zebra chip disease in potato. The pathogen is vectored by the tomato-potato psyllid *Bactericera cockerelli* to potato, tomato, and other solanaceous plants. Translocation patterns of Lso in tomato and susceptible and potentially tolerant varieties of potato were examined to determine whether rate or direction of translocation vary by host species or potato varieties. Two insects were given a 7-day inoculation access period on a single leaf. Weekly, leaves from different locations on the plant were tested for the presence of Lso by PCR. In tomatoes and potatoes, Lso was detected 2 to 3 weeks after infestation, most frequently in the younger leaves (located at the top and middle of the plant). In potato, the pathogen also was detected in leaves on a second stem, not treated with insects, when the stems remained joined via the tuber. Although the patterns of pathogen movement throughout the plants were similar among potato varieties, symptoms developed earlier in more susceptible varieties. Quantitative PCR indicated that bacterial titers were frequently low in tomato and potato samples (<20 genome units/ng DNA),

highly variable temporally and spatially, and not necessarily correlated with symptom severity. Results establish that for improved detection, leaf sampling should include newly-developing leaves and consider the potential delay between infection time and when titers are sufficiently high for PCR detection.

**Association of phytoplasmas and rod shaped bacteria with citrus disease of unknown etiology in the state of Baja California Sur, Mexico**

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Multiple symptoms of yellow-type diseases were observed in citrus trees from various citrus growing areas in the state of BCS. Leaf malformations, chlorosis,irregular blotching and necrotic spots, yellowing of leaf veins and shoots, necrotic leafstalks and dryed shoots, leaf dropping and dieback - symptoms reported for distinct citrus diseases worldwide, related with phytoplasmas and other vascular pathogens. To determine the possible causal agent/agents of citrus maladies, samples from symptomatic lemon, sweet orange, mandarin and grapefruit trees were collected and processed for scanning electron microscope (SEM) analysis. Specimens from leaf midribs, leafstalks, young leaflets and stems from symptomatic plants were prepared by original technique reported earlier and observed in Hitachi S-300N SEM. Both phytoplasmas and rod shaped bacteria revealed in phloem tissue of diseased plants. The most abundant concentration of phytoplasmas was detected in the samples of mandarin (south of BCS) where bacteria were not detected. On the contrary, in samples of lemon and orange trees collected from northern BCS along with phytoplasmas, some rod-shaped bacteria were observed with specific smooth outer surface. In some sieve tubes their concentration was very high. Phytoplasma size ranged from 500 to 1000 nm, and bacterial sizes reached 3000x 500 nm, similar to former RLO sizes reported in the case of citrus greening. A very high level of phytoplasma (but not rod shaped bacteria) infection was registered also in acacia species, as well as in some herbaceous plants growing in the same plots, which suggests wild hosts of this pathogen. In some lemon and orange samples phytoplasma detection was fulfilled also by nested PCR technique using phytoplasma specific primers. The work is in progress to confirm all the cases of phytoplasma infection detected by SEM, to identify both pathogens and prove the possible mixed infection of two pathogens to determine the correct disease etiology.

**Field trials of phytopathogenic fungi as an alternative to control the invasive weed, *Hyparrhenia rufa***

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*Hyparrhenia rufa* is an aggressive weed commonly known as Jaragua grass or horsetail, which has invaded croplands in Puerto Rico. In order to reduce the need for herbicide applications, we explored the use of phytopathogenic fungi as a strategy for its control. During 2009, plants showing foliar symptoms were collected from the Lajas UPR-Agricultural Research Station. *Curvularia* sp. *Fusarium* sp., *Sphaeropsis* sp. and *Phoma sorghina* were found to be pathogenic to *H. rufa* under greenhouse conditions: Two field trials were conducted during August and December 2011. Plants were evaluated at two different growing stages by height: young (61 to 91 cm) and mature plants (122 to 195 cm). Fungal conidial or mycelial suspension grown on potato dextrose agar were used as inocula. Sterile distilled water was used as control. ANOVA for a randomized complete block design was used to compare fungal pathogens potentials as biological control agents. During the first trial conducted in August 2011 using mature plants, lesions were small, fluctuating from 0.6 to 1.1 cm. There were no significant differences ($P > 0.05$) in lesion size among treatments with the exception of *Curvularia* sp. and *Sphaeropsis* sp., the later being more virulent. In the second trial using younger plants, lesion size ranged from 1.0 to 20 cm, and significant differences were found ($P < 0.05$) among fungal treatments. *Fusarium* sp. was the most virulent compared to the other fungal species evaluated, causing larger lesions on foliage. Further studies will include specificity of isolates, as well as the evaluation of multiple-pathogen use strategy.

**Standard and High Fidelity PCR to detect *Pythium dissotocum* in hydroponically grown cilantro (*Coriandrum sativum* L.)**

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*Pythium* root rot is a recurrent problem in hydroponically grown cilantro (*Coriandrum sativum* L.) in Puerto Rico. Cilantro plants showing severe stunting and root rot were obtained from Adjuntas, Puerto Rico. Tissue sections were placed in potato dextrose agar (PDA) and water agar (WA) amended with antibiotics. *Pythium* sp. was isolated from diseased tissue. Pathogenicity tests were conducted incorporating 10 g of *Pythium* sp. inoculum grown on pearl millet in soilless substrate (Promix). Two-week-old cilantro seedlings cv. 'Lone Star' and pea seeds (*Pisum sativum* L.) cv. 'Sugar Baby' were sown in 20 individual pots and eight pots where included as controls. Twenty-one days after inoculation small necrotic spots were visible on the lower portion of the stem and on the tap root of cilantro seedlings. Necrotic lesions were evident on pea seedlings fifteen days after inoculation. Standard and High Fidelity PCR were carried out to determine the sensitivity and specificity of both methods in the detection of *Pythium* sp. from diseased tissue. Conventional and Hi-Fidelity PCR was performed on DNA extracted from two isolates (1271 and 1272) using the ITS1/ITS4 primer pair. Sequencing of the internal transcribed spacer region of nuclear ribosomal DNA indicated 99% homology with *Pythium dissotocum*. The pathogen was isolated from diseased tissue and characterized based on morphological, cultural and molecular characteristics using both conventional and Hi-Fidelity PCR to confirm Koch's postulates. Early detection of the pathogen can be crucial to implement management practices in hydroponic systems.

**Identification and characterization of *Ralstonia solanacearum*, the causal agent of bacterial wilt of tomato**

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Bacterial wilt in tomato caused by *Ralstonia solanacearum* was detected in June 2010 in Coamo affecting about 3,000 tomatoes (*Lycopersicon esculentum*). New outbreaks occurred in orchards in Aguada and Lares. Symptoms observed were necrotic buds and wilting of the plant. *Ralstonia solanacearum* was identified after isolations on nutrient agar (NA), semi-selective triphenyl tetrazolium chloride agar (CTZ) and DAS ELISA (Pathoscreen Kit, Agdia, Indiana, U.S.A.). Conventional and Hi-Fidelity PCR products with primers 759 and 760 amplified a 280 bp band of the 16S rRNA region. The sequencing results indicated 100% homology with *Ralstonia solanacearum*. Isolates 1287 and 1290 were identified as biovar 1 for the production of acid from carbohydrates: dextrose, trehalose, lactose, maltose, cellobiose, mannitol, sorbitol, and the reduction of nitrite from nitrate. Pathogenicity tests on tomato seedlings cv. 'Beefsteak' using a bacterial concentration of $10^{-8}$ CFU/ml, showed wilting and necrosis of the vascular bundles seven days after inoculation. The DAS ELISA serological test and comparatively conventional and high-fidelity PCR with primers 759 and 760 proved that *Ralstonia solanacearum* caused the wilt symptoms in tomatoes. Conventional and Hi-Fidelity PCR detected *R. solanacearum* from vegetal tissue of symptomatic plants. Hi-Fidelity PCR did not detect the bacteria in four tissue samples while Conventional PCR detected *Ralstonia solanacearum* in all samples analyzed. This disease poses a threat to tomato production on the Island and early identification is essential to prevent spread to other solanaceous species.

**Genetic diversity of '*Candidatus* Liberibacter asiaticus' from Southeast Asia and South America**

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Huanglongbing is one of the most serious diseases threatening citrus production. The bacterial disease produces inedible fruit and shortens the lifespan of infected trees. Three species of the bacteria cause the disease, but *Candidatus* Liberibacter asiaticus is the most wide-spread, found throughout Asia, South America and North America. To better understand the genetic diversity of *Ca*. L. asiaticus, a concatenated dataset of 3 conserved genes was used to create a multi-locus sequence typing scheme, a phylogeny and for genetic diversity analyses. Forty-five strains of *Ca*. L. asiaticus from 7 countries in Asia and South America were sequenced and used in the analyses. An eBURST analysis of the strains placed 31 of the strains into clonal complexes. One complex included primarily Thai strains, while the second complex contained Chinese, Thai and Brazilian strains. The majority of the Brazilian strain did not place in a cluster. The neighbor-joining phylogenetic tree constructed from the same dataset indicated 4 groups with some similarities to the eBURST analysis. The Chinese and Thai strains exhibited similar patterns of clustering between the methods, but the phylogenetic analysis brought resolution to the South American strains with two groups of Brazilian strains recovered. Genetic diversity estimates also indicated that Brazilian strains exhibited more diversity than all the strains from Asia which may indicate increased selection pressure in the new environment. There was also no geographic differentiation among the strains or genetic differentiation between strains of different host origins. These results indicate that multiple introductions have occurred in Brazil possibly from countries throughout Asia.

**Ecological niche assessment of the causal agents of Huanglongbing and its vectors**

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Huanglongbing, caused by three phloem-limited bacteria *Candidatus* Liberibacter ssp., is a devastating disease of citrus vectored by the two psyllids. *Ca.* L. asiaticus and its vector the Asian citrus psyllid *Diaphorina citri* Kuwayama are the largest geographic distribution of the bacteria and psyllid, respectively. The spread of *Ca.* L. africanus and its vector the African citrus psyllid *Trioza erytreae* Del Guercio has been limited to the African continent and Middle East. Previous work indicated that the African citrus psyllid is heat-intolerant, preferring temperatures less the 30°C, restricting the distribution of both. To understand the ecological forces shaping the different distributions, the geographic ranges of the two bacteria and two psyllids was gathered from the literature and government reports. Nineteen variables including annual mean temperature and annual precipitation were examined for their effects on the bacterial and psyllid distributions. Fine scale ecological assessment was performed using the maximum entropy algorithm MaxEnt with Akaike information criteria to determine which climatic variables are influencing the distribution. The ecological niche for *T. erytreae* can be described by 3 variables that account for 49.2% of the distribution, while six variables explain 63.5% of the range of *D. citri*. The geographic distributions of *Ca*. L. africanus and *Ca*. L. asiaticus can be explained by 4 variables accounting for 70.3% and 7 variables accounting for 80.5% of the niches, respectively. Precipitation of the coldest quarter had the best predictive power for the distributions of *Ca.* L. asiaticus and *D. citri*. The niches of *Ca.* L. africanus and *T. erytreae* were best explained by two different climatic variables, temperature seasonality for the bacteria and temperature annual range for the vector. The similarity in niches between *Ca.* L. asiaticus and *D. citri* may indicate a stronger co-evolutionary relationship compared to *Ca*. L. africanus and *T. erytreae.*

**The population structure and possible origin of the plant pathogen *Pseudomonas syringae* pv. *actinidiae***

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Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) was first described in Japan in the 1980's. The disease was later observed in several other regions around the world including Korea, Italy and most recently New Zealand. This disease is now the major limiting factor of kiwi production in Italy, Japan and Korea, and is viewed as an emerging threat in the United States. To better understand the progression and pathogenicity of the pathogen, the population structure of 24 isolates of Psa, 1 isolate of *P. syringae* pv. *thea* and 2 isolates *P. syringae* pv. *syringae* were collected from Italy, Greece, Japan and Korea. In addition to the four housekeeping genes used in the P. syringae typing scheme, 12 other housekeeping genes were also sequenced in order to evaluate any population structure in the pathovar. Because housekeeping genes typically have little variation within a pathovar, three additional hypervariable regions, outer-membrane proteins, were also sequenced. The 12 new housekeeping genes had no variation present and were excluded from the analysis. The four genes from the typing scheme were used to create a concatenated dataset with 69 other pathovars of *P. syringae* including 28 more strains of Psa. In the resulting phylogeny, all Psa strains grouped together with some strains *P. syringae* pv. *thea* occurring within the group and others diverging outside the group. This indicate that Psa may have been derived from strains of *P. syringae* pv. *thea*, a pathogen of the tea plant. In China where *P. syringae* pv. *thea* occurs, tea plants and kiwi trees occur side-by-side making a host shift possible. Finally, the three omp genes sequenced indicate more diversity with Psa, but no population structure.

**The persistence of *Gliocephalotrichum bulbilium* and *G. simplex* causing fruit rot of rambutan in Puerto Rico**

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Fruit rot of rambutan (*Nephelium lappaceum* L.) is a pre and post-harvest disease problem that affects fruit quality. Significant post-harvest losses have occurred worldwide and several pathogens have been identified in Malaysia, Costa Rica, Hawaii, Thailand, and Puerto Rico. In 2011, fruit rot was observed on rambutan at the USDA-ARS Tropical Agriculture Station in Mayaguez. Infected fruit sections (1mm2) were surface sterilized, rinsed with sterile deionized-distilled water and transferred to acidified potato dextrose agar (APDA). Isolates of *Gliocephalotrichum* spp. were recovered from diseased fruit tissue and diferentiated based on morphological characteristics such as color, texture of the colonies, and the production of reproductive structures. In 2008, this genus had also been observed on rambutan. Twenty-six isolates were transferred to carnation leaf agar for morphological, molecular and pathogenicity characterization. Using light microscopy, scanning electron microscopy, and PCR amplification of the ITS1-5.8S-ITS2 region of the rDNA and β-tubulin partial cds gene, 13 isolates were clearly divided into *G. bulbilium* (*Gb*) and 13 into *G. simplex* (*Gs*). Sequence analyses gave 100% identity with these two species. Morphologically, *Gb* was identified by the presence of bulbilloid aggregates and stipe extensions that were mostly located adjacent to the start of the conidiogenous penicilli. For *Gs*, conidiophores had stipe extensions rising at some distance away from the conidiogenous penicilli, with no bulbilloid aggregates but with chlamydospores that were unicellular, brown, smooth, and thick-walled. Pathogenicity tests were conducted on healthy superficially sterilized fruits. Fruits were inoculated with 5-mm mycelial disks of 8-day-old pure cultures grown in APDA. Untreated controls were inoculated with APDA disks only. Five days after inoculation (DAI), white mycelial growth for *Gb* and golden mycelial growth for *Gs* were observed on fruits. Eight DAI, fruit rot symptoms were observed on both isolates of *Gb* and *Gs* and conidiophores were observed on spintems (hair-like appendages).

***Pantoea ananatis*, a maize plant pathogenic bacterium associated with chlorotic streaks and rolling upper leaves**

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Symptoms of chlorotic streaks, leaf blight and vascular wilt has have recently reported on maize caused by *Pantoea agglomerans* in the Central Highland Valley of Mexico. In addition to these symptoms rolling upper leaves were consistently observed in maize from commercial and experimental plots in Montecillo and Chalco, Estado de Mexico, during 2007 to 2011. To identify and characterize the causal agent involved with this symptom, portions of 0.5 cm2 tissue from areas considered both healthy and diseased were excised, disinfested and placed on Casamino acid and King's B media. Petri dishes were incubated at 28°C and after 48 h, cream and yellow colonies were observed. Seventy isolates were selected for physiological and biochemical tests. Results indicated that these isolates were Gram negative, and nonfluorescent on King's B medium. For DNA analysis, 1.5-kbp fragments of the 16S rRNA gene were amplified and sequenced with universal primers. Results of the phylogenetic analysis grouped the isolates in *P. ananatis*, and *P. agglomerans* clusters. Isolates CPO-8, CPO-12B, CPO-27B, CPO-47 and CPO-95 of *P. ananatis* were selected for pathogenicity tests. Symptoms of water-soaked lesions were observed 4 days post-inoculation followed by chlorotic to straw-colored leaf streaks. Rolling upper leaves were observed at 5 weeks post-inoculation. In addition, those these isolates were infiltrated at 107 CFU mL-1 into tobacco leaves and a hypersensitive response was observed. Isolates were reisolated, and the 16S rRNA gene fragments were 100% similar to their original isolates sequences of *P. ananatis*. This result provides information about this new symptom in maize that should be taking into consideration in breeding for resistance to diseases caused by *Pantoea* species.