2014 Caribbean Division Meeting Abstracts

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Reaction of *Musa* hybrids and cultivars to Colombian isolates of *Mycosphaerella fijiensis*

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Black leaf streak disease (BLS), caused by *Mycosphaerella fijiensis*, is the most important foliar disease of *Musa* spp. Six *Musa* hybrids and 10 cultivars were screened for their resistance to six *M. fijiensis* isolates, under greenhouse conditions. Inoculations were made on 6-month-old plants and carried through a virulence assay using weighed fragmented mycelia brushed onto delineated areas on the abaxial surfaces of the first and second fully unfolded banana leaves. Grand Naine and FHIA-23 were used as susceptible and partially resistant controls, respectively. The disease progress over time was assessed at each inoculation site using a qualitative scale, obtaining the following variables: infection degree (ID), incubation period (IP) and time of evolution of symptoms (TES). Disease severity was also rated at the end of experiment (day 84 after inoculation). Correspondence analyses were performed to characterize the cultivar-isolate interaction in terms of evaluated variables, and the resistance groups were categorized by cluster analysis. BLS symptoms were produced in all *Musa* accessions and were observed at 21 days after inoculation. There was significant differences ($P < 0.001$) between isolates for disease severity, where virulence levels were determined using the mycelium-based assay. Considering only the most virulent isolates, the hybrids FHIA-23, FHIA-17, FHIA-18 and the cv. Saba were the most resistant accessions (i.e. higher IP and TES, and lower DS and ID), whereas the Grand Naine and Kelong Mekintu were the most susceptible cultivars. The other hybrids (FHIA-03, FHIA-01 and FHIA-25) and cultivars (Fougamou, Maritu, Pelipita, Prata, Pysang Ceylan, Red Yade and Yangambi km5) showed low to moderate levels of partial resistance. *Musa* resistance to Colombian isolates of *M. fijiensis* were confirmed and could be a valuable tool for the integrated pest management of *M. fijiensis* by reducing the pathogen pressure on plantain and banana fields.

Nutmeg wilt and mortality in Grenada and its connection to endemic *Phytophthora* species

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A survey of *Phytophthora* was initiated during March 2011 in the Island of Grenada. In total 16 streams were selected across the island. Foliage of tropical forest trees in the Grand Etang National Park was also surveyed to discover foliar *Phytophthora*. In addition, three nutmeg sites were surveyed for involvement of *Phytophthora* spp. with nutmeg wilt and mortality. To isolate *Phytophthora* from streams, 1–2 month old mango leaves were deployed using mesh bags and collected two days later. One-week-old mango leaves were used as bait when isolation was attempted from soils that were collected underneath wilted nutmegs. Isolation from tree foliage was attempted by selecting leaves with dark necrotic lesions that have recently dropped or still were attached to the plant. For all isolations, 1–3 mm sections of necrotic tissue were cut and dried using filter papers and plated on V8 Juice based PARPNH selective media. Outgrowing colonies subcultured after two days and were identified by combination of morphological features and molecular classification. In total, 678 isolates (449 *Phytophthora* and 229 *Pythium*) were obtained. These included *P. capsici*, *P. heveae*, *P. macrochlamydospora*, *P. nicotianae*, *P. palmivora*, *P. tropicalis* and nine hitherto unknown species. *Phytophthora palmivora* and an unknown species closely related to *P. katsurae* (99.1% sequence match) was isolated from stem and root lesions of wilted nutmeg trees. *Phytophthora macrochlamydospora* was isolated from soil of two trees at one location. While all known and unknown species were recovered from streams, foliar *Phytophthoras* included only *P. heveae*, *P. palmivora*, *P. tropicalis* and four of the unknown species. *Phytophthora palmivora* was reported to cause seedling mortality and trunk canker on nutmegs, however its involvement along with the new species found in this survey should be evaluated to reveal the primary pathogen involved with common nutmeg wilt in Grenada.

Identification and molecular characterization of pigeon pea witches'-broom phytoplasma in plants and its potential vectors in Puerto Rico

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Few studies have determined the presence of phytoplasma from important crops in Puerto Rico. Typical phytoplasma symptoms were observed in different plant species such as pigeon pea (*Cajanus cajan*), periwinkle (*Catharanthus roseus*), tabebuia (*Tabebuia pallida*), Spanish lime (*Melicoccus bijugatus*), jungle geranium (*Ixora coccinea*), mango (*Mangifera indica*), cacti (*Opuntia* spp.), citrus trees (*Citrus* spp.), and coffee (*Coffea arabica*). Sixty two plant samples from these species were tested using end point PCR with universal and specific primers (i.e., nested PCR) that amplify the 16S rDNA and ribosomal protein genes (*rplV-rpsC*) genes. Fifty one

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percent of the samples tested corresponding to periwinkle, pigeon pea, citrus, coffee and tabebuia were positive for phytoplasmas with amplicons of 0.8 and 1.2 kb, respectively, depending upon the primers used for PCR. In both cases, the DNA sequences and restriction patterns for RLFP obtained from these samples showed 99% of identity with pigeon pea witches'-broom phytoplasma (PPWB) belonging to group 16SrIX. Due to the lack of studies of potential insect vectors, common *Auchenorrhyncha* species were sweep-collected from pigeon pea and citrus and tested for phytoplasma. Of nine insect genera collected, *Empoasca kraemeri*, *Melornemis antillarum* and *Colpoptera maculifrons* were positive for PPWB phytoplasma using conventional PCR and DNA sequence analysis. These findings indicate that these insects feed upon the aforementioned plant species, and may act as potential phytoplasma vectors in the field. Finally, specific primers were designed for qPCR assay to amplify a 102-bp region of the 16S rDNA gene from samples with low level infections of phytoplasma. By the SYBR® Green method, the melting temperature (T_m) recorded in positive samples was 82.3°C. These primers amplified and identified DNA of phytoplasma belonging to the groups and subgroups 16SrV-A, 16SrIII-H, 16SrII-D, 16SrV-C, 16SrII-C, 16SrVI-A, 16SrXII-A and 16SrIX-C.

3-dimensional modeling of protein structures distinguishes closely related phytoplasmas

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Phytoplasmas (formerly mycoplasma-like organisms, MLOs) are cell wall-less bacteria that inhabit phloem tissue of plants and are transmitted from plant-to-plant by phloem-feeding insects. Numerous diseases affecting hundreds of plant species in many botanical families are attributed to infections by phytoplasmas. Phytoplasmas are classified in 16S rRNA gene (16S rDNA) RFLP groups and subgroups that to date embrace over 35 '*Candidatus* Phytoplasma' species. However, closely related strain and species lineages can be difficult to distinguish based on conserved gene sequences such as 16S rDNA. In this work, we studied the deduced amino acid sequences of selected proteins encoded by the genomes of phytoplasma strains and compared the 3-dimensional structures the proteins modeled *in silico*. Closely related strain lineages, as well as distantly related strains, were clearly distinguished by differences in protein structural models. The results indicated that some amino acid positions in a protein may reflect lineage differences more clearly than do others, and that 3-dimensional modeling can provide useful means for distinguishing closely related strains that may differ in biological properties.

Evaluation of *Phaeoisariopsis griseola* on common bean *Phaseolus vulgaris* in Puerto Rico

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Angular leaf spot (ALS), caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris sin. *Pseudocercospora griseola* (Sacc.) Crous & U. Braun., is an important disease in common bean (*Phaseolus vulgaris* L.) in the Caribbean and Central America. The wide pathogen variability makes it necessary to continuously monitor virulence patterns when breeding common bean for resistance. A greenhouse and field experiments were carried out to determine the reaction of 12 ALS differentials, 19 genotypes, and local check 'Verano' to the pathogen in Juana Diaz, on the southern coast. Prevalent warm and wet conditions in the months of March and April were ideal for disease development. In the greenhouse, the experiment was arranged in a completely randomized design replicated four times and inoculated with isolate ISA-1-2010 (1×10^5 conidia ml⁻¹). In the field trial, the 20 genotypes were arranged in a randomized complete block design with three repetitions, and the differential lines were included in a single row with 10 plants each. In the field, genotypes were evaluated with natural ALS infection. The evaluation was carried out 12 days after inoculation in the greenhouse and at the R2 growth stage in the field, using the CIAT scale where: 1 to 3 = resistant, 4 to 6 = moderately resistant, and 7 to 9 = susceptible. Under field conditions, disease severity was higher than in the greenhouse. Lines G11796, G-5686, "Flor de Mayo" and "Montcalm" were susceptible to *P. griseola*. Amendoin, Bolón Bayo, Don Timoteo, Amendoin, Verano, Mexico 54, Cornell 49242, Pan 72 and G2858 were moderately resistant. BAT 332 was resistant. Lines ADP-010, ADP-132, ADP-143, ADP-158, ADP-160, ADP-161 and ADP-429 were resistant to *P. griseola*. Moderately resistant lines were ADP-106, ADP-154, ADP-006, ADP-213, ADP-225 and "Verano". In the greenhouse, the isolate ISA-1-2010 produced similar results with the Mesoamerican and Andean genotypes.

Management strategies, associated fungi and intensity of guava speck in Michoacán, Mexico

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Guava speck is a major disease of guava fruits in Michoacan, Mexico. This disease has been associated with nutritional deficiencies and fungi. The objectives of this research were to evaluate different disease management strategies based on chemical and biorational fungicides, identify the associated fungi and to determine the losses associated with the disease in three producing regions in the state of Michoacán. In 2011 and 2012, three and two experiments were established, respectively, in commercial orchards of guava. Fungicides were applied every 14 days from green to ripe fruits. In 2011, strategies were based on benomyl, azoxystrobin, pyraclostrobin, thiophanate-methyl and thia-bendazole in blocks alternated with captan and copper oxychloride, quaternary ammonium salts and *Bacillus subtilis*. In 2012, 13 strategies similar to the previous year were evaluated including plant resistance inducers Glutathione-oligosaccharins and potassium phosphite. The results in each season showed significant differences between treatments ($P < 0.0001$). The programs beginning with pyraclostrobin or azoxystrobin alternated with captan and / or copper oxychloride in blocks of 2 applications of the same active ingredient, reduced the incidence and severity of guava speck significantly in relation to the control. Glutathione-Oligosaccharins and potassium phosphite showed intermediate levels of control. *Bacillus subtilis* and strategies based on quaternary ammonium salts and hydrogen dioxide showed no significant difference with the control. The associated fungi, based on PCR amplification and sequencing of the ITS region, indicated 99 to 100% similarity with sequences deposited in the Genbank corresponding with *Pantospora guazumae* and *Mycosphaerella aurantia*. The data indicated yield losses of more than 11% associated with the disease. The results show a significant effect of management strategies in the intensity of guava speck, which could be associated with fungi. Although the pathogenicity tests have not been completed until now, treatments have contributed significantly to the reduction of production losses.

Identification and characterization of *Colletotrichum* spp. affecting yam (*Dioscorea* spp.) crop in Puerto Rico

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Yam anthracnose, caused by *Colletotrichum gloeosporioides*, is widely distributed in most yam-producing areas worldwide, causing losses of up to 90% in the most palatable species, *Dioscorea alata* and *D. rotundata*. To increase yam production in Puerto Rico, the objectives of this research were to determine disease severity throughout the island and identify and characterize *Colletotrichum* species associated to yam. Fungal isolates obtained were characterized based on morphology, pathogenicity and with two sets of molecular markers (ITS and β -tubulin). Two hundred twenty four isolates of fungi were collected from the germoplasm collection located at the Agricultural Experiment Station in Corozal, Puerto Rico and from yam production fields throughout the island. Of these, 43 isolates were characterized as *Colletotrichum* spp. showing different growth rates and phenotypes on selective media. Using the ITS region one isolated of *D. alata* (cultivar PI 15587) was identified as *Colletotrichum gloeosporioides*. Nine other genera of fungi were also identified. The majority of *Colletotrichum* isolates were identified from *D. alata*, specifically from cultivars PI 15587, Kabusach, and Gunung. Koch's postulates confirmed the pathogenicity of *Colletotrichum* spp. on *D. alata* and *D. rotundata*. The most severely affected by anthracnose was the Barranquitas municipality with a 70% of disease severity on *D. rotundata* followed by the municipality of Moca with 50% disease severity on *D. alata*. These results are of significant importance to establish reliable control measures to increase yam production in the region.

Identification of fungi in field rice and their sensitivity to fungicides

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Field rice plants at Campeche, Mexico, presented symptoms such as spots on stems, leaves and brown grain discoloration. Grains after polished, may cause dark kernels, thus reducing their market value. This study identified fungi present in rice and determined their sensitivity to fungicides. Plant samples with symptoms were collected from wetland fields and tissues from leaves, stems and grains, were then disinfested. Tissues or seeds were placed on water agar plates or blotter to fungi and incubated at 25°C. Pure cultures were transferred to PDA medium. Based on the morphological characteristics, 14 different fungi genera were identified. *Fusarium*, *Curvularia*, *Bipolaris*, *Papulospora* and *Thielaviopsis* were found in stems, leaves and grains. *Nigrospora* was found in stem and leaf samples. *Nakataea* and *Phoma* were found in stems tissues. *Cladosporium*, *Rhizopus* and *Aspergillus* were present in leaves only. *Microdochium* was seen in stems and grains. *Pyricularia* and *Rhizoctonia* were found in grains only. Sensitivity to fungicides *in vitro* tests were performed with 13 fungicides at commercial doses for *Fusarium* and *Curvularia*. Three fungicides (benomil, azadirachtin and carbendazim) reduced more than 95% of mycelial growth for *Fusarium* and two fungicide treatments (Cupric oleate and Cymoxanil + Mancozeb) presented the same mycelial reduction for *Curvularia*. Field tests showed that of the best eight of 13 fungicides tested *in vitro*, kasugamicin reduced 94% grain discoloration incidence. At harvest, grains from the eight fungicides treatments showed the presence of the fungi *Fusarium*, *Curvularia*, *Aspergillus* and *Bipolaris* at different percentages. This study documented the incidence of different fungi genera in rice field plants and showed grain discoloration reduction level with commercial fungicides.

History of *Citrus leprosis virus* recorded in herbarium specimens

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Citrus leprosis is the common name of a virus disease of citrus in which necrotic local lesions are produced on citrus and the virus is transmitted by mites in the genus *Brevipalpus*. This disease is at the same time an emerging, a current and a historical disease problem. For some time, researchers have understood that the *Citrus leprosis virus* (CiLV) occurs in two distinct forms, one that produces particles only in the nucleus of infected cells (CiLV-N; *Dichoravirus*) and another, much more widespread type, that produces particles in the cytoplasm of infected plant cells (CiLV-C, *Cilevirus*). Both forms produce bacilliform particles with bipartite, single stranded RNA genomes. CiLV-C is widespread in South America and can be found as far north as parts of Mexico. The distribution of CiLV-N is much more restricted, having been reported only in Brazil, Panama, Mexico and very recently, in Colombia. Neither form of the virus occurs in the USA today, although Citrus leprosis disease was a problem for Florida citrus prior to the mid 1960's, at which time the disease disappeared for reasons that are not well understood. We have recently used degradome sequencing to show that there is great diversity within both the CiLV-N and CiLV-C viruses. The USDA ARS maintains a collection of disease samples from citrus that were taken to document interception of restricted pathogens at border inspection stations. This includes more than 50 samples identified at the inspection stations 50 or more years ago. We have applied NGS degradome sequencing to some of these samples identified as Citrus leprosis. We have obtained near full-length genome sequences of both a typical CiLV-C strain intercepted from Argentina in 1967 and another CiLV-N strain obtained in Florida in 1948. The latter is a completely novel form of CiLV-N, not known to exist anywhere in the world today.

Fungi and Oomycete pathogens associated with pepper wilt in Michoacán Mexico

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Pepper wilt significantly reduces yield in peppers (*Capsicum annuum*) inducing farmers to change crops or abandon farm lands that are infested with the associated pathogens. Although in general *Phytophthora capsici* has been associated to the disease in producing regions from central Mexico, more plant pathogens have been associated, but their interactive effects on the disease severity are unknown. The objective of this research was to evaluate the co-existence of these pathogens and the development of disease symptoms in pepper. In order to determine the fungi/oomycete associated to pepper wilt, a survey from July to November of 2013 was conducted in Yurecuaro, Vista

Hermosa and Tanhuato municipalities in Michoacán Mexico. Isolations were done on V8 and PDA media. From 200 samples processed, 32% corresponded to *Fusarium oxysporum* and *Fusarium solani*, 29% to *Phytophthora capsici* 24% to *Rhizoctonia solani*, 4% to *Pythium* and 11% to pathogens not previously identified associated with pepper wilt. Identification at the molecular level through total DNA extraction from fungi using the method of 2% CTAB was performed. Amplifications of the internal transcribed spacer (ITS) region of the rDNA were performed, using primers ITS5 and NL4 amplifying approximately 1100bp. The amplified fragments were verified by horizontal electrophoresis and sequenced using Sanger methodology. The pathogens *F. oxysporum*, *F. solani*, *P. capsici*, *R. solani*, *Pythium* sp. and *Plectosphaerella cucumerina* were identified after the sequences were compared with those deposited in the GenBank database. This is the first time that *Plectosphaerella cucumerina* has been identified associated with pepper wilt in Michoacán, Mexico. This novelty will inform future management strategies for pepper wilt in this region.

Monitoring of endangered plant species at the karst region of Puerto Rico to detect plant pathogenic threats

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Forests at the northern and southern karst of Puerto Rico enclose more than 20 endangered plant species. A diversity of biotic factors such as pest and diseases are usually overlooked as potential precursors of final extinction events of these plants. Thus, this research pursues the evaluation of 23 endangered plant species in 8 forest of the karst region to detect plant pathogenic threats. Plant populations were evaluated in term of health, viability, reproduction, quantity of individuals and, associated plant pathogens. At each location, symptomatic tissue from leaves, fruit, flower or stem was collected for further analysis. Fungi and bacteria were isolated in respective selective media and identified using morphological and molecular criteria. The most frequently isolated fungi were: *Colletotrichum* spp., *Pestalotia* spp., *Curvularia* spp., *Phomopsis* spp., and *Botryodiplodia* spp. All of these fungi were isolated from several of the 23 endangered plant species at both karst regions. Pathogenicity test conducted in *Stahlia monosperma* ("cobana negra") confirmed that *Colletotrichum* spp. and *Pestalotia* spp. are able to infect the plant with and without lesion causing necrotic spots on leaves. In *Ottosulshia rhodoxylon* ("palo de rosa"), *Eugenia woodburyana* and *Banara vanderbiltii* ("palo de ramón") only *Pestalotia* spp. were able to directly penetrate plant tissue. *Colletotrichum* spp. and *Botryodiplodia* spp. were less virulent causing necrotic lesions in these two plant species. Two out of the seven bacteria strains isolated were gram negative. One strain was identified as *Pseudomonas* sp. that colonized *Calyptronoma rivalis* ("palma manaca") inflorescence. To our knowledge this is the first report of these plant pathogens affecting endangered plant species in Puerto Rico. These data will be useful to develop protocols to accomplish the conservation of these plant species.

A real-time PCR assay to detect and quantify 16SrIII-L and 16SrI phytoplasmas associated with cassava frogskin disease in Costa Rica and Paraguay

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Cassava has the ability to grow on marginal lands, it tolerates drought, and it can grow in low-fertility soil. Several diseases, including cassava frogskin disease (CFSD), threaten the sustainability of cassava production. Recently, this disease was discovered to be associated with infection by phytoplasmas of groups 16SrIII-L and 16SrI. The disease is exponentially propagated through asexual seed, creating a demand for disease-free planting materials. Two TaqMan® probes were designed for the microorganisms, based on the *rp* gene (16SrIII-L phytoplasma) and 16S rRNA gene (16SrI phytoplasma). We used qPCR to obtain a sensitivity that was 100- and 1,000-fold higher than that obtained from nested PCR. In Paraguay, phytoplasma belonging to groups 16SrIII-L and 16SrI was detected in the localities of Caaguazú, Isla Sola, and Barrientos Cue. In Costa Rica, phytoplasma from group 16SrIII-L was detected in the localities of Aguas Zarcas, Pital, Los Lagos, La Merced, Los Chiles, Alajuela, Fortuna, and Guápiles. The CFSD symptoms observed in Paraguay were less severe than those observed in Costa Rica, where phytoplasma group 16SrIII-L showed greater disease severity. With the qPCR technique, both phytoplasmas could be detected and cassava planting certified disease-free, thus allowing germplasm exchange.

Educational strategies to promote plant pathology at public schools and at the University of Puerto Rico

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Our goal as educators is to radically change student's perceptions of agricultural sciences as a backward field, towards a new paradigm of agriculture constantly led and improved by new technological advancements. Techniques based on DNA analysis, immuno- and nanotechnologies are newer ways to study food- and plant-microbe interactions. Sponsored by a USDA's NIFA/HSI grant we have implemented different educational strategies that had directly impacted public schools and university students in their understanding and use of complex cellular and molecular concepts in plant pathology. So far, through the organization of 8 science clubs ("Club de Agro-Microbios") we have impacted 108 students from public schools of the Western region of PR. We have increased the number of skilled students, faculty and technicians in the application of DNA-based, immuno and nanotechnologies to solve phytopathological problems at University of Puerto Rico (UPR). This has been achieved by laboratory instrumentation acquisition by which these novel technologies are integrated in various undergraduate and graduate courses, and in students' research projects of the crop protection program at UPR. In addition, a summer workshops series focused in the applications of bio- and nano-technologies in plant pathology have introduced students, faculty and technicians to modern technologies used for rapid diagnosis and management of DNA sequence data of plant pathogens, while providing fundamental principles in plant pathology. We have increased the numbers of highly educated alumni in areas related to crops and food biosecurity. Acquired knowledge will have a major impact in the success of new Hispanic scientists through their training in new applications, and their improved capability to formulate and solve new questions related to crop biosecurity and food safety of tropical agricultural production systems. The ultimate goal is to improve the quality of life of more than 900 underrepresented Hispanic minorities in Puerto Rico.

Fungal pathogen complexes associated with diseases of rambutan, longan and mango in Puerto Rico

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Different fungi have been associated with diseased inflorescences, leaves, and fruits of mango, rambutan and longan. During a fungal disease survey conducted between 2008 and 2013 at six orchards of rambutan and longan, and one orchard of mango in Puerto Rico, symptoms such as fruit rot, inflorescence blight, rachis canker, flower and vascular necrosis, flower abortion and leaf blotch were observed and sampled. Diseased tissues were disinfested and plated onto APDA medium. A total of 1143 fungal isolates were isolated and identified morphologically and by DNA sequencing of the ITS1-5.8S-ITS2 region of the rDNA, partial region of the 28S large ribosomal subunit, and fragments of β -tubulin gene, histone H3, calmodulin, actin, chitin synthase, glyceraldehyde-3-phosphate dehydrogenase and trans elongation factor 1-alpha (EF1- α). A total of 262 (22.7%) isolates belonged to the Botryosphaeriaceae, 172 (15%) as *Diaporthe* spp., 128 (11.2%) as *Pestalotiopsis* spp., 112 (9.8%) as *Lasmenia* sp., 93 (8.1%) as *Fusarium* spp., and with lesser frequency, *Gliocephalotrichum bulbilium*, *G. simplex*, and *Calonectria hongkongensis*. Pathogenicity tests confirmed that fruit rot of rambutan was caused by a complex of pathogens such as *Lasmenia* sp., *Lasioidiplodia* sp., *Colletotrichum* sp., *C. hongkongensis*, *G. bulbilium*, *G. simplex*, *Pestalotiopsis* sp. and *Diaporthe* sp. Four fungal species in the Botryosphaeriaceae (*Lasioidiplodia theobromae*, *Neofusicoccum* sp., *N. parvum*, and *N. mangiferae*) caused inflorescence blight in mango. *Lasioidiplodia theobromae* also caused inflorescence blight and fruit rot of longan. *Lasioidiplodia* sp. caused leaf blotch and inflorescence blight of

rambutan. *Albonectria* sp. caused inflorescence wilt, vascular and flower necrosis in rambutan, longan and mango. *Diaporthe pseudomangiferae* caused inflorescence rot, rachis canker and flower abortion of mango. Fungi, such as *Guignardia mangiferae*, *Cladosporium cladosporioides*, *Nigrospora oryzae*, *Alternaria alternata*, and *Xylaria* spp., were endophytes on these fruit crops. Knowing the pathogens involved in each disease complex will allow for better plant disease management practices.

Pathogenic, morphological and molecular characterization of (*Phytophthora* spp., causal agent of cacao black pod disease in Puerto Rico

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Cacao (*Theobroma cacao* L.) production is concentrated in West Africa, and Central and South America. In recent years its production has increased significantly exceeding an annual growth of 2.2% according to FAO in 2010. The final report of the "Global Industry Analysts, Inc." stated that cacao production will reach 4.8 million tons by 2015. Worldwide this crop is affected by diseases that drastically reduce its production. Among them, cacao black pod caused by (*Phytophthora palmivora*, with losses that exceed \$400 million annually. The aim of this study was to characterize three (*Phytophthora* spp. isolates on the basis of morphology, DNA analysis and pathogenicity. Isolates were obtained from symptomatic cacao pods of clones HY 27418, UF 122 and SIAL 56, collected at Tropical Agriculture Research Station (TARS) germplasm collection located in Mayaguez, Puerto Rico. Oomycetes isolates were cultivated on acidic PDA, PARPH and V8 agar. Isolates were identified by light microscopy using taxonomic keys. Criteria such as sporangia shape, size and prominence of papillae, as well as chlamydospore production were examined. PCR amplification of cytochrome c oxidase gen (COXI) was used for molecular characterization of isolates. Pathogenicity tests were conducted on healthy cacao fruits, inoculated with one ml of sporangia (1×10^4 sporangia/ml). (*P. palmivora* was identified using morphological characters. To confirm our findings COXI gen was amplified and sequenced from an isolate obtained from clone SIAL56. DNA sequence has 99% homology with (*P. palmivora* GenBank accession number: AB688308. Four days after inoculation, isolate obtained from SIAL 56 recorded the highest disease severity per fruit (50.93%) compared to the other two isolates obtained from clones UF 122 (30.82%) and HY 27418 (29.76%).

Evaluation of the tepary bean (*Phaseolus acutifolius*) CIAT germplasm collection for response to Bean Common Mosaic Necrosis Virus (BCMNV)

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Aphid-transmitted Bean Common Mosaic Necrosis Virus (BCMNV) and Bean Common Mosaic Virus (BCMV) are potyvirus that cause production losses in bean. Pathotype groups correspond with resistance genes that can be organized in two groups. One group has resistance conferred through recessive isolate-specific genes with five alleles, *bc-1*, *bc-12*, *bc-2*, *bc-22*, and *bc-3* at three loci that need *bc-u* for expression. The second group has resistance conferred through the dominant *I* gene. Developing resistance to viruses will be critical for expanding tepary bean production in areas where they have a high incidence. This study evaluated the response of 207 genotypes from the CIAT (Centro Internacional de Agricultura Tropical) tepary collection to BCMNV using a glass house. The inoculum of the NL3 strain was prepared using BCMNV infected leaves in cold 0.05 M phosphate buffer, pH 7 (aprox. 1:10 w/v). One week after emergence the primary leaves were inoculated. Cultivar 'Verano' (which has the *I* gene) was used as a control. Veinal necrosis and local pinpoint necrotic lesions were identified as a response to BCMNV. Genotypes G00041, G00042, G00044 and G40177E1 showed consistent symptoms in three inoculations, with veinal necrosis on the inoculated leaves suggesting the presence of the *bc-12* gene. Genotype G40177E showed restricted local pinpoint necrotic lesions, which in *P. vulgaris* indicates the presence of *I* protected by the *bc-22* gene, however the SW13 SCAR marker did not function in tepary. G40001, G00078, G40177A2, G40269 showed an inconsistent inoculated response.