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Region-wide aflatoxin reductions for improved food security, income, and health

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Crop infection by several *Aspergillus* species is associated with accumulation of aflatoxins. Aflatoxins are genotoxins that retard human development, impair immune systems, reduce animal productivity, prevent access to markets, and cause cancer. Fungi that cause contamination are distributed throughout warm environments in crops, insects, uncultivated plants, soil, and air. Aflatoxins form in crops beginning during plant development and continuing through crop maturation, harvest, and storage, with crops remaining vulnerable until consumed. Human exposure occurs either upon consumption of contaminated crops and products of animals fed contaminated crops or when small crop fragments and fungal spores are respired. A simple biological control alters fungal populations so that aflatoxin producers are scarce and the quantities of aflatoxins in crops, and throughout the environment, are reduced to below levels of concern. Biocontrol products that use atoxigenic genotypes of *A. flavus* as active ingredients have been effective on millions of hectares of maize, groundnut, pistachio, fig, and cottonseed. Commercial use in the developed world has shown the safety of fungal communities improving both on treated crops and throughout production areas. Atoxigenic strain based products are also produced in developing countries and are a simple, low tech method for improving both human health and access of agricultural products to markets. Applications take advantage of natural fluctuations in magnitude and composition of fungal communities with treatment influences extending across areas and between seasons with cumulative benefit. Lessons from this type of biocontrol may be applicable to other plant disease problems.

75 years of potato cyst nematodes in the United States: A case study for quarantine pests

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Quarantine pests pose a significant threat to the agriculture economy of the United States. Potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*, are globally important nematode parasites of potato and both are considered quarantine pests in most countries. In the United States, *G. rostochiensis* was detected in New York in the 1940s and *G. pallida* was more recently detected in Idaho in 2006. In addition to these detections, a new species of *Globodera*, *G. ellingtonae*, was described from populations collected in Oregon and Idaho. The regulatory stories of *G. pallida* and *G. rostochiensis* in the United States provide a framework in which to evaluate the pros and cons of regulatory and management/eradication practices used to contain these economically important plant-parasitic nematodes. More recently, GLOBAL (Globodera Alliance) was formed and represents the only comprehensive effort in the U.S. to tackle the threat posed by invasive *Globodera*. The goals of GLOBAL, including use of genomic approaches to characterize pathogen virulence, identification of potato germplasm with resistance to three species of *Globodera*, and development of science-based approaches to deal with the threat of *Globodera* will be discussed.

New approaches to control postharvest rot in fruits

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Postharvest fungal diseases are one of the main factors causing losses of fresh produce in the warm and humid tropical regions. Levels of postharvest losses are affected by type of commodity, country, weather condition, production practices, infrastructure, transportation environment or equipment, and refrigeration facilities. Fungal pathogens penetrate host tissues by direct breaching of the host cuticle or through wounds caused by biotic or abiotic agents in the field or in storage, and through natural openings such as lenticels, stem ends and the fruit pedicel. An integrated approach for postharvest disease management should include cultural preharvest and postharvest practices. Postharvest diseases are often controlled by the application of synthetic fungicides, however, to reduce postharvest losses with minimal use of fungicides, other alternatives such as plant bioactive compounds, biological control agents, generally recognized as safe (GRAS) products, and physical treatments have been investigated in papaya, mango, banana, and pineapple fruits. Integration of two or more of such alternatives to control pathogens could improve management of fruit postharvest diseases.

Main phytosanitary problems in the countries of the OIRSA region

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During the year 2016, the global phytosanitary problems were HLB in citrus (*C. Liberibacter* sp.), and species of fruit flies. At the continental level, the quick decline syndrome of olives (*Xylella fastidiosa*), and the tomato leaf miner (*Tuta absoluta*) in Europe and Africa. Coffee rust (*Hemileia vastatrix*) in Latin America once again caused one of the most important epidemics that drastically affected production, economy and social stability in producing countries. In the American Continent, the most important quarantine pests are *Fusarium oxysporum* f. sp. *cubense* 4TR, CBD (*Colletotrichum kahawae*) in coffee, and the bean kernel Khapra beetle (*Trogoderma granarium*). In addition to these pests, in 2015–2016 crops in the OIRSA region were affected with asphalt spot of corn, geminivirus-whitefly complex attacking solanaceae and staple crops. Tospovirus transmitted by thrips attacking vegetables, and banana sigatoka were the main problems in terms of the number of chemical applications. Scientific societies and professionals play an important role in plant health; especially in the fields of phytosanitary surveillance, pest risk analysis, development of endemic and quarantine pest lists, dynamic phytosanitary risk maps, pest early warning systems and prediction models, specialized technical groups, harmonization of diagnostic techniques, phytosanitary emergencies, pest prospecting for trade facilitation, regional regulation, preparation of technical manuals, virtual trainings; among other areas in the production and operation chains of the Ministries of Agriculture.

Fusarium tropical race 4 and it threat to food security and the banana industry in Latin America and the Caribbean

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Bananas mean food security and livelihoods for millions people in Latin America and the Caribbean (LAC). About 20 million tons of bananas (64% of production) are locally consumed in LAC and seven countries of the region belong to the top-10 exporting nations. Pest and diseases are major challenges for this crop, especially Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). In the 1950s, Foc race 1 disrupted the banana industry forcing the replacement of the susceptible cultivar Gros Michel for the resistant Cavendish. While Cavendish plantations remain unaffected by FW in LAC, a new strain, tropical race 4 (TR4), has destroyed more than 100.000 ha of this cultivar in Asia and has recently spread to the Middle East and Africa. TR4 is a quarantine disease and risk analyses suggest that its spread to LAC is matter of time. TR4 not only affects Cavendish, but many other local banana cultivars important for food security. No resistant cultivars to replace these varieties are currently known. Therefore, exclusion is the top priority. While traveler alerts and contingency plans are in place in some countries of LAC, building capacities in growers and services providers on FW symptoms and epidemiology-based management strategies, is still a major challenge. In this work we present comprehensive data about the threat poses by TR4 for LAC giving directions towards exclusion, preparedness and research priorities. Countries are encouraged to engage coordinately on regional exclusion and preparedness initiatives and to strongly invest on innovative and solutions-oriented research programs.

Distribution and frequency of the phytopathogenic nematodes associated with potatoes (*Solanum tuberosum* L.) in the Province of Cartago, Costa Rica

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Potatoes are an important source of income for Costa Rican farmers and Cartago is the province with the most area dedicated to this crop (around 78% of the total area cultivated with potato). Plant parasitic nematodes causes yield reduction and tuber quality deterioration on potato. Information about the frequency and distribution of the nematode populations is necessary to implement effective management strategies but is lacking in Costa Rica. The objectives of this research were to identify the genera and to determine the frequency of the plant parasitic nematodes associated with potatoes in Cartago, as well as to determine the distribution of the three most important potato-parasitizing nematode species. To achieve these goals, soil and root samples were taken from 25 potato fields (from 1609 to 2918 m.s.l.). Nematode genera were identified by their morphological characters, and counted under a microscope. For root samples the most frequent genus was *Pratylenchus* (63%), followed by *Tylenchus* (37%), *Globodera* (33%), *Meloidogyne* (30%), *Helicotylenchus* (28%), *Aphelenchus* (26%), *Aphelenchoides* (22%), *Hemicycliophora* (9%) and *Gracilacus* (2%). For soil samples, the most frequent nematodes were *Globodera* (58%), followed by *Pratylenchus* and *Tylenchus* (52%), *Meloidogyne* (40%), *Helicotylenchus* (38%), *Aphelenchus* and members of the family Criconematidae (27%), *Hemicycliophora* (23%), *Aphelenchoides* (19%), members of the family Trichodoridae (15%), *Ditylenchus* (10%) and *Xiphinema* (4%). Currently, the *Globodera*, *Meloidogyne* and *Pratylenchus* species are being identified using molecular techniques and phylogenetic approaches, as well as the intraspecific variability. This information will be valuable for breeding and crop rotation programs.

Diagnostic method to identify Fusarium wilt pathogens in ornamental palms

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Fusarium oxysporum ff. spp. *palmarum* (FOP) and *canariensis* (FOC) cause Fusarium wilt on certain ornamental palm species. To date there are 2 palm hosts of FOC and 5 palm hosts of FOP. Traditional detection and identification of FOP involves PCR amplification and sequencing with EF-1α primers. The goal of this research was to design an efficient diagnostic method to differentiate palm Fusarium wilt pathogens from other *Fusarium* species often associated with palms. A TEF based primer pair (FPW) was designed using NCBI Primer BLAST. To validate the specificity of FPW primer set, 75 known isolates of FOP (18), FOC (18), *F. solani* (3), *F. proliferatum* (5), *F. concentricum* (3), *F. incarnatum-equiseti* species complex (4), *F. sacchari* (2) and non-pathogenic *Fusarium oxysporum* (16) were selected and PCR was performed using three different sources of polymerases. The FPW amplicon yielded ~ 569 bp, and all amplicons were sequenced. All FOP and FOC isolates, which had been obtained from symptomatic petiole or rachis tissue, were amplified. Sixteen of the non-pathogenic *F. oxysporum* yielded an amplicon. However, 50% of these 16 isolates were recovered from roots and trunk. No other *Fusarium* species were amplified with FPW primers. Results indicate that symptomatic petiole or rachis is the only tissue to be used for isolation of palm Fusarium wilt pathogens. Confirmation of the specific forma specialis (FOP vs FOC) will still require sequencing the FPW amplicon.

Characterization of *Phytophthora capsici* in Taiwan using a universal host differential

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Phytophthora capsici (Leon.) causes significant losses to pepper production in Taiwan and worldwide. Our objectives were to better understand the race structure of *P. capsici* within a single season and develop a standardized system of race characterization using a host differential. Isolates were collected from important pepper growing regions of Taiwan from May to August 2016. In total, 27 isolates were characterized for virulence using the New Mexico Recombinant Inbred Lines and the World Vegetable Center host differentials. In addition, all isolates were genotyped for 63 polymorphic single nucleotide polymorphism loci using a targeted sequencing approach. Based on the host differential, 24 isolates were identified as new races. Additionally, a subset of 10 host differentials were able to efficiently characterize the races in Taiwan. The isolates in Taiwan were found to have a relatively narrow genetic base, be primarily triploid and of the A2 mating type. This work provides a basis for *P. capsici* race characterization on a more global scale and may help plant breeders and pathologist limit losses due to *Phytophthora* blight.

Genetic characterization and incidence of whitefly borne-begomoviruses in vegetable production in Costa Rica

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Begomoviruses have emerged as important plant pathogens in tropical and subtropical regions worldwide. While these viruses were reported during the 1970’s in Costa Rica, their current diversity and distribution are poorly known. The objective of this study was to analyze the incidence and diversity of begomoviruses in commercial tomato and sweet pepper crops from different agricultural production systems of the major growing regions of Costa Rica. Samples were randomly collected from both greenhouse and field production systems during 2011 and 2012 in three different geographical locations: Cartago, Grecia and Zarcero. The presence of

bipartite begomoviruses *Tomato yellow mottle virus* (ToYMoV), *Tomato leaf curl Sinaloa virus* (ToLCSiV), and *Pepper golden mosaic virus* (PepGMV), and the monopartite begomovirus *Tomato yellow leaf curl virus* (TYLCV) was confirmed in samples using hybridization, rolling circle amplification (RCA) and full length genome sequencing. Virus incidence varied from 0 to 26% depending on the region and host species considered. In Cartago, only PepGMV was detected, with an incidence of 18.2% in sweet pepper samples. Similarly, only ToYMoV was detected in Zarcero on tomato, with a high incidence (25.8%). In Grecia, TLCSiV, ToYMoV and TYLCV were detected on tomato plants although in all three cases, incidence was low.

Passiflora-Xanthomonas **pathosystem studies**

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Passiflora edulis has long attracted attention due to its economic value that lies in the production of passion fruit juice, an essential exotic ingredient in juice blends. Brazil is the main producer of passion fruits, and leaf spot, caused by *Xanthomonas axonopodis* (Xap) is an important disease that attacks orchards. Our goal is to understand the mechanisms that underlie the defense responses during passion fruit-Xap interaction. Firstly, we constructed two cDNA libraries, and several plant transcripts expressed in response to Xap were identified. A set of these genes was analyzed using qPCR, and results indicated a lipoxygenase 2 (500× and 300×, 5 and 9 dai, respectively) and a neomenthol reductase (3.3× and 8.1×, 5 and 9 dai, respectively) in plant defense. ***P. alata***, a second cultivated species is also susceptible, and qPCR results showed a consistent pattern of expression (5 dai) in 9 selected genotypes from a full sib progeny. Lipoxygenase 2 and neomenthol reductase were differentially induced in two distinct genotypes '136', (3.5×) and '49' (20.5×), respectively. In parallel, studies using the same progeny (100 individuals) found 20 QTLs with response to Xap, of which 9 related to leaf necrosis and 11 to injury. The individual effects ranged from 0,2% to 15,7%, and two large-effect QTLs (R^2 = 15,7%) were assigned to linkage groups (III and IV) of the linkage map of sweet passion fruit. Functional marker development based on expressed transcript sequences aiming at fine gene mapping is now being conducted in our laboratory.

Colletotrichum fructicola, **causal agent of avocado anthracnose in Central Mexico and its management with antifungal compounds**

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The anthracnose disease, the most common fungal avocado postharvest diseases, is caused by species of Colletotrichum. Different strategies are used to reduce this infection during avocado production; however, the disease is still prevalent in some regions of Mexico. The aim of the present study was to identify the causal agent of anthracnose in avocado produced in Central Mexico and to evaluate its susceptibility to thiabendazole and biological control treatments such as *Bacillus* spp. or methanol extracts obtained from *Phytolacca icosandra*, *Prosopis laevigata*, and *Sapindus saponaria*. Isolates of *Colletotrichum* spp. were recovered from infected fruits and subject to DNA extraction, PCR amplification (ITS region, ACT, GAPDH, CHS and ApMat genes), DNA sequencing, Multilocus Sequences Typing (MLST) analysis using Bayesian inference. The analysis revealed that *Colletotrichum fructicola* was the species associated with anthracnose in avocado produced in Central Mexico. Also, it was shown that *Bacillus* sp. had the highest inhibitory effect (78%), against *C. fructicola* follow by ethanol extracts from *P. laevigata* (65.24%), *P. icosandra* (43.49%) and *S. saponaria* (32.49%). Importantly, all of the selected *C. fructicola* isolates were resistant to thiabendazole. Taken together, these results provide new information to design strategies to control anthracnose disease in avocado production.

Plant-parasitic nematode associated to corm rot disease of apio (*Arracacia xanthorrhiza* Bancroft) in Puerto Rico

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Apio (*Arracacia xanthorrhiza* Bancroft) is a starchy crop produced in Puerto Rico. The crop is originally from the highlands of South America, and it has been adapted to grow in the mountainsides of Barranquitas and Orocovis towns. During the last 10 years, apio production and cultivated area have decreased due to the "corm rot disease" that causes yield losses and contamination of the propagative material. The symptoms include wilt and yellowing of the plant. Our objective was to identify the main causal agent of apio corm rot disease. In the field, the disease presents a spatial distribution typical of phytonematodes, therefore we collected soil from four affected commercial farms to evaluate the presence of nematodes. From the total nematode species found, *Rotylenchulus reniformis* presented an 89.0% of frequency and free-living nematodes a 0.9%. *R. reniformis* is one of the most important plant-parasitic nematodes limiting plantain and banana production, crops that are used by the farmers in rotation with apio and could increase nematode populations in the soil. We found in one of the farms an average of 387 larvae per 100 cm^3 of soil. Our results suggest that the phytonematode makes injuries in the corm that later are colonized by a complex of fungi and bacteria producing the rot. Most of fungi and bacteria, identified in the soil had no ability to directly infect the tissue. This is the first report of *Rotylenchulus reniformis* associated as the main causal agent of apio corm rot disease in Puerto Rico.

Detection of palm infecting phytoplasmas by digital PCR: Technical aspects and application

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Phytoplasmas belonging to the 16SrIV taxonomic group have historically caused large-scale losses to palms in the Caribbean basin, particularly in coconut production. Currently, palm-infecting phytoplasmas are a significant threat to the sustainability of palm production in the region. Some of the challenges to understanding the epidemiology of the diseases caused by associated strains is the inability to culture phytoplasmas in the lab, the rapid onset of symptoms and subsequent decline of infected palms, and difficulty in vector discovery. Technical challenges include difficulty in sampling palms and obtaining sufficient quantity of phytoplasma from tissue for molecular research. Limitations to detecting low levels of phytoplasmas has been reduced with the advent of qPCR which has allowed for earlier detection of phytoplasma in infected plants, being able to detect the disease agent prior to symptom onset, while traditional PCR fails to detect phytoplasma until palms show symptoms. Another tool that is only beginning to be adapted to plant pathology research is digital PCR which increases sensitivity 5-fold over qPCR. Additionally, this technology drastically reduces the impact of inhibitors present in reactions as a result of extraction techniques. In this study we have developed the first TaqMan assay used for digital PCR to detect 16SrIV-D phytoplasmas. The assay functions for 16SrIV-A phytoplasmas and likely for all strains in the 16SrIV group.

Laurel Wilt: The story of how one beetle has wiped out millions of Lauraceae trees and the promising research that may stop its spread

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Laurel wilt (LW) is a relatively new vascular wilt disease which kills trees in the Lauraceae plant family, including numerous ecologically and economically important trees such as avocado (*Persea americana*) and redbay (*Persea borbonia*). To date over one-third (300+ million) of the redbay population alive before the laurel wilt invasion have succumbed to the disease and US avocado production, valued at more than US$400 million, is threatened. Since its introduction in 2002, LW has spread as far south as Miami and as far west as Texas, moving faster than anticipated. LW is caused by a fungus *Raffaelea lauricola* that is vectored by its symbiont the redbay ambrosia beetle, *Xyleborus glabratus*. *R. lauricola* causes a systemic vascular wilt disease in affected trees. The beetle feeds off the fungus which it "farms" inside its host. It is suspected that the interaction of the fungus with the host causes rapid tylosis production, which impedes water flow through the xylem resulting in crown wilt and death of the plant. Management of this disease is impeded by evidence that the damage caused by just one beetle may be sufficient to initiate infection. To date, preemptive fungicide applications have been found effective in protecting trees, however the prohibitive expensive limits its wide-spread application. This presentation will cover new areas of research including the use of volatile-based repellents, coppice-management, and host tolerance. Potential impacts on avocado production in Central America will be highlighted.

Emergence and impact of viral diseases vector-borne in Cuban agriculture

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The emergence or re-emergence of virus vector-borne affecting agriculture is increasing a previously unknown rate, with a serious impact on agriculture yields and the environment. In recent years, new viruses have appeared in Cuba, mainly transmitted by whitefly and thrips, with severe losses in many economically important crops. Several surveys conducted in the last five years have permitted identified begomoviruses and toposviruses species in productive and non-productive ecosystems. Tomato chlorosis spot virus was identified for the first time in the country, affecting tomato, tobacco, pepper and common bean transmitted by *Frankliniella shultzei.* Another species of begomoviruses transmitted by whitefly were detected in common bean and soybean named Tobacco leaf curl Cuba virus, Common bean severe mosaic virus, Common bean mottle virus and Rhynchosia golden mosaic Yucatan virus. Also new species of begomovirus have been identified in wild plants as *Rhyncosia* sp, *Euphorbia* sp, *Sida* sp, and *Malvastrum* sp. Molecular studies of the whitefly have confirmed the presence of *Bemisia tabaci* MEAM1 species in both agricultural and non-productive ecosystems coexisting with the native species New World (NW). The use of genetic resistance is currently the most important strategy diseases control and helps to reduce environmental impacts, aspect in which plant pathologists and geneticists worked together in order to improve the breeding programs.

BIO-PCR for *Pseudomonas* spp. detection in soybean (*Glycine max*) seeds

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Soybean has become one of the most widely consumed foods in the world. However, bacterial diseases are one of the main factors that limit this crop, affecting yields and seed quality. A strategy to avoid these infections could be early detection and elimination of contaminated seed lots. Some bacterial genus have been detected in seeds by BIO-PCR, a technique that allows to increase bacterial concentration and has greater sensitivity than conventional PCR. In Cuba, even when soybean is imported to produce milk, oil, yogurt and beef; the crop has not been generalized. However, it is important to have disease diagnosis systems. The aim was to evaluate a BIO-PCR system to detect *Pseudomonas* genus in soybean seeds. Generic primers were designed and specificity was evaluated. Pseudomonas strain isolated from soybean was diluted in seed extracts obtained by incubation overnight and sensitivity of the BIO-PCR system using generic primers was evaluated. The PCR using PgB1 and PgB2 generic primers was highly specific to detect *Pseudomonas* genus. Using BIO-PCR was possible to detect bacterial strain until 10^2 UFC*ml^{-1}. The BIO-PCR system is useful to detect *Pseudomonas* spp. in soybean seeds, allowing early detection of pathogens which could affect this crop.

Characterization of Fusarium species from pineapple plantations in Costa Rica

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Pineapple monoculture, mainly the hybrid MD2, is highly susceptible to different pathogens, including *Fusarium* spp. In Costa Rica, since approximately 2000, producers have observed an increase of a symptom that appears as a yellowing on the leaf tips and is known as “amarillon”. The disease is characterized by drying out of the leaves, loss in vigor and severe lesions such as necrosis and decay of the vascular system. No visible symptoms have been observed on the outside of fruits, but internally the tissue is sunken with necrotic lesions. To determine which organisms were associated with this problem, 101 samples were collected from plantations from the main production areas, including the Northern zone, as well as the Caribbean area of Costa Rica (Alajuela, Limón and Heredia provinces). Symptomatic and asymptomatic portions of tissue were used to isolate the microorganisms. Sixty-two samples (63%) yielded at least one Fusarium species, while on the other 39 (37%) samples, bacteria and other fungi were observed. Translation elongation factor-1a (TEF) genes were used to sequence 116 isolates of Fusarium. Results showed: *F. solani* (1 isolate), *F. incarnatum* (1 isolate), *F. polyphialidicum* (8 isolates), *F. oxysporum* (22 isolates) and 84 isolates of *F. ananatum*. Of the 116 isolates, 19 were isolated from fruit (18 were identified as *F. ananatum*) and 97 were isolated from other parts of the pineapple plant. Genetic analysis and pathogenicity tests are currently being performed.

Rapid isothermal detection of *Xylella fastidiosa* through recombinase polymerase amplification

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Xylella fastidiosa (*Xf*), living and multiplying in the xylem network, is an important quarantine pathogen. *Xf* is of America origin, but in recent years has appeared in Italy, France, Germany, and Spain. The genetic diversity indicates that these new introductions are independent of one another. Therefore, a rapid, sensitive, and reliable detection method is critical to reduce the likelihood of *Xf* introduction into a new region. Agdia has developed a rapid isothermal assay for specific detection of *Xf* using the advanced recombinase-polymerase amplification technology. The assay performs both as a real-time and an endpoint test from a single reaction tube at 39°C for 20 minutes. Reaction template is simply prepared by soaking 50 mg of petiole cross-sections in 0.5 mL AMP1 extraction buffer for 10 minutes. The assay reacts to over 27 isolates from grapevine, citrus, olive, almond, coffee, oleander, mulberry, American elm, sycamore, oak, blueberry, and blackberry while consistently detecting 22 copies of spiked *Xf* genome in petiole extract (1:10, W/V). No reaction background was observed in host tissue such as grapevine, citrus, olive, almond, coffee, blueberry, and blackberry. No cross-reaction was observed to *Xanthomonas*, *Erwinia*, *Pseudomonas*, and *E. coli*. This assay provides users a fast and reliable tool to monitor *Xf* regional and international movements.

Identification of *Erwinia billingiae* as a causal agent of bacterial canker on mango (*Mangifera indica*) trees in Costa Rica

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Bacterial canker of mango (*Mangifera indica*) emerged during the 1970s in Venezuela and it was named as “fruit spot”. The causal agent of this disease has been identified by conventional phenotypic approach as *Erwinia mangifera, Erwinia herbicola* and *Pectobacterium* in the past 30 years. In Costa Rica, this disease was observed in the mid 1980s, and continue to cause economic losses

in most production areas of the country. In order to confirm the identification of the causal agent, we carried out a polyphasic characterization. Pathogenicity of the bacterial isolates was assessed on the Tommy Atkins variety by inoculating fruits and trunks with a 1×10^7 cfu/ml suspension of each isolate, and incubation under greenhouse conditions at 25–30°C. Isolates causing the same symptoms were subjected to API 20E and API 50CHE (bioMérieux) and GN microplates (BIOLOG) identification systems as recommended by the manufacturer. To address the genealogy of this bacterial pathogen, molecular characterization was performed by extracting DNA using DNeasy Blood & Tissue Kit (Qiagen) and PCR amplification of 16S rRNA, recA, gyrB, dnaN, gltX and rpoB gene fragments. Sequences were obtained and a phylogenetic tree was constructed using Bayesian inference with reference bacterial sequences. API and GN (BIOLOG) microplates failed to identify the bacterial isolate as *E. herbicola* or *Pectobacterium carotovora* as previously reported, however the phylogenetic analysis of 16S rRNA and concatenated MLST genes placed the Costa Rican strain closely related to *Erwinia billingiae* species.

An updated list of plants associated with the foliar nematodes and the potential of mtCOI for *Aphelenchoides* species identification

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Fourteen *Aphelenchoides* species have been recognized as plant-parasites in a broad range of plants; the most important species, i.e. the foliar nematodes *A. besseyi*, *A. fragariae* and *A. ritzemabosi*, have been reported on more than 1000 associated-plant records. To appraise the potential host range that a single or a specific combination of *Aphelenchoides* spp. could have, we compiled a comprehensive dataset of the associated plants for these 14 species, based on available literature and online databases. The data were plotted on an associated-plants' supertree, in combination with an *Aphelenchoides* consensus phylogeny. The combination of low interspecific and high intraspecific morphological variability makes morphology-based identification of *Aphelenchoides* species extremely difficult. Informative genetic sequences are usually restricted to the small and large subunits of rDNA (D2D3 and 18S regions) while the region that is being used as the standard barcode for almost all animal groups, the mitochondrial Cytochrome Oxidase I gene (COI), remained largely unexplored. To tackle this gap, we generated 196 new aphelenchid sequences (39 of which were COI regions), including the main plant-parasites of the genus. Compared to the rDNA markers, the COI had similar K2P distances and an alike success rate for PCR amplification. We also constructed the first concatenated analysis for this genus.

Identification of plant parasitic nematodes associated with weeds in potato (*Solanum tuberosum* L.) fields in the province of Cartago, Costa Rica

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Potato (*Solanum tuberosum*) is the main vegetable cultivated in Costa Rica and Cartago is the main production area of this crop. Nematodes cause significant reductions in yield and on tuber quality. A common cultural practice in potato fields in Costa Rica is to leave the field fallow and allow the establishment of a wide diversity of weeds that maintain the nematode populations. The objective of the research was to identify the plant parasitic nematodes associated with weeds on potato farms in Cartago. A total of 250 samples of different weeds were collected from several localities in the north of Cartago, such as San Juan de Chicuá, El Convenio, Llano Grande, Tierra Blanca and Pacayas. Nematodes in the genera *Pratylenchus*, *Helicotylenchus*, *Meloidogyne* and the family Heteroderidae were detected at a relative frequency of 85, 36, 34 and 32%, respectively. *Meloidogyne*, *Pratylenchus* and nematodes of the Heteroderidae family were found associated with the weeds *Brassica campestris*, *Gallinsoga quadriradiata*, *Lepidium virginicum*, *Melampodium perfoliatum*, *Poa annua*, *Polygonum aviculare*, *P. persicariodes*, *Richardia scabra*, *Rumex obtusifolius* and *Spergula arvensis*. *Meloidogyne* and *Pratylenchus* were found in association with *Bromus* spp., *Chenopodium album*, *Juncus bufonius* and *Plantago australis*, while *Pratylenchus* and nematodes of the Heteroderidae family were identified in *Capsella bursa-pastoris* and *Calceolaria mexicana*. *Meloidogyne* was also identified in *Cyperus* spp., whereas *Pratylenchus* was found in *Lolium* sp., *Senecio vulgaris*, and *Sonchus oleraceus*. Currently, the species of *Pratylenchus*, *Meloidogyne* and Heteroderidae associated with these weeds are being identified using morphological, molecular techniques and phylogenetic approaches.

Root-lesion nematode Pratylenchus alleni: Distribution, host plant and effect of temperature in Quebec, Canada

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In Canada, the root-lesion nematode *Pratylenchus penetrans* is the predominant species in agricultural soils and causes significant yield losses in horticultural crops. In 2011, *P. alleni* was first observed in Canada in a soybean field in the province of Quebec, which caused over 50% yield losses in damaged areas. Based on soil samples recovered from 185 soybean fields and 10 agricultural regions of Quebec, *P. alleni* and *P. penetrans* were found in 8 and 45% of the samples respectively, while both species were present in 5% of the samples. In greenhouse trials, host range study of *P. alleni* revealed that corn, potato, soybean and pearl millet could increase

populations to harmful levels. Based on greenhouse competition trial on soybean, *P. alleni* reproduction was not significantly modified in presence or absence of *P. penetrans*. The optimum temperature for *P. alleni* reproduction was found to be higher than *P. penetrans*.

Molecular characterization and distribution of the needle nematode *Longidorus laevicapitatus* Williams, 1959 (Nematoda: Longidoridae) in Costa Rica

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Correct identification of *Longidorus* species in Costa Rica is essential to establish appropriate control strategies for preventing the spread of these nematodes. Nematode surveys conducted in the rainy seasons from 2013 to 2015 in areas arbitrarily chosen and widely distributed in the whole territory of Costa Rica, resulted in an overall prevalence of *Longidorus* spp. infesting soils both cultivated, ornamental and wild plants of 40.26%. Integrative morphometric and molecular data for *Longidorus* populations were obtained using D2-D3 expansion segments of 28S rRNA, ITS1-rRNA, and the partial 18S-rRNA, identifying a solely species identified as *Longidorus laevicapitatus*. Morphology and morphometrical traits analysis of these populations of *L. laevicapitatus* were in agreement with those of the original and posterior descriptions of the species, except for some minor differences, which may be a result of intraspecific variability. The Phylogenetic relationships of this species with other representatives of *Longidorus* spp. using D2-D3 expansion segments and the partial 18S indicated that *L. laevicapitatus* clustered clearly separately in a basal position in both phylogenetic trees.

Efficacy of fungicides for powdery mildew on pumpkin in NW Texas was dependent on disease pressure and host susceptibility: 2009–2014

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In the Texas High Plains, characterized by hot temperatures and dry weather, powdery mildew (PM) on irrigated pumpkins used for ornamental purposes, is a constant disease problem, especially on susceptible varieties. In studies conducted since 2009, fungicides were tested alone or in combination with others for the management of PM. In 2009, with the susceptible ‘Oz’, fungicides sprays containing the labeled rate for cyflufenamid sprayed twice and alternated with triflumizole during the season had the best control, with yields 52.9% greater than the untreated control. Some fungicide applications containing myclobutanil or pyraclostrobin and boscalid also were not statistically better than the control for that season. Fruit numbers were significantly higher with cyflufenamid alternated with triflumizole (58.4%) and with quinoxyfen alternated with cyflufenamid. In 2010, the onset of PM on ‘Oz’ occurred later in the season and no fungicide applications, including those containing cyflufenamid or quinoxyfen, were statistically better at managing PM, nor having significantly higher yields or fruit numbers. From 2011 until 2014, the use of varieties with good resistance to PM such as ‘Summit’ and ‘Orangita’ fungicide applications for the management of PM have had no significant impact on yield nor fruit number, as any PM was at low to trace levels by the end of the season.

Identifying resistance to cavity spot in carrots

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Cavity spot of carrot is caused by several *Pythium* species, especially *Pythium violae* and *P. sulcatum*. Field trials were conducted from 2013 to 2016 to screen 60 carrot lines from the USDA breeding program for differences in susceptibility to cavity spot. Several cultivars were included as commercial checks. Carrots were seeded into naturally infested ‘muck’ soil (pH 5.7- 6.5, organic matter 65–78%). In 2016 carrots were also grown in mineral soil in California. After harvest, carrots were washed and assessed for cavity spot incidence and disease severity (DSI). Cavity spot incidence and severity were very high in 2013 (97% and 69 DSI) and 2014 (96%, 54 DSI) and lower in 2015 (50%, 26 DSI) and 2016 (30%, 17 DSI). Disease incidence was high in the California trials (up to 91%). A wide range of susceptibility to cavity spot was found. Purple lines 6139B, 6244B, 7262B and 3497B were highly resistant (1–8%, 1-7 DSI). Two orange carrot lines, in the trials in 2014 to 2016, had consistently low disease. Line 1137B had 8–9% cavity spot with DSI of 4-7. Line 5367 B had 5–21% cavity spot, with a DSI of 3 - 8. Cv. Atomic Red was highly susceptible in all trials. Maverick and Envy had high cavity spot in California and Ontario, while Upper Cut had relatively low disease, and Cellobunch was moderately susceptible at both sites. Field screening has identified breeding lines with promising levels of resistance to cavity spot.

Targeting the bad and the ugly Pseudomonas spp. causing bacterial diseases in mushroom production

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Although bacterial diseases of mushrooms have been studied for more than 100 years, producers continue to encounter major problems during mushroom production, mainly because there are no consistently effective management methods for these bacterial diseases in this crop. The focus of this research is on bacterial diseases caused by the genus *Pseudomonas* as they are the most economically important pathogenic bacteria in mushroom production and management requires taking into account non-target effects on beneficial pseudomonads. The ultimate goal of this research is to develop management strategies for bacterial diseases of mushrooms caused by *Pseudomonas* species. It is imperative to understand the genetic diversity of pathogenic *Pseudomonas* species that cause disease in mushroom in order to develop controls (biological and other) that are specific for the pathogens. Multilocus Sequence Analysis (MLSA) was used to describe the diversity of bacterial pathogens causing disease on cultivated mushrooms. Sequence-based techniques allowed for identification of organisms at the species level, ecotype, and strain (sequencing type). Mushroom samples were obtained from symptomatic mushrooms from different rooms and farms. Kings B medium amended with novobiocin, penicillin, and cycloheximide was used to specifically isolate *Pseudomonas* species. All the isolates were screened using repetitive element PCR (rep-PCR) and two to three representative strains of each genomic fingerprint were selected for further study. Preliminary MLSA data indicate that as many as seventeen different genotypes and at least six different species exists.

Mycotoxins contamination of different grains in Costa Rica

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Aflatoxins (AFs) and fumonisins are mycotoxins that represent a threat for human and animal health. In Costa Rica, total aflatoxin limits are 20 μg kg^{-1} for all cereals and legumes, 15 μg kg^{-1} for peanut, and for fumonisins, there are no regulatory limits established. Most imported agricultural commodities are subject to AFs monitoring, however, information is scarce in terms of the level of mycotoxins in local sources of grains. Therefore, the objective of this work was to summarize the results of the aflatoxin analyses conducted in the Mycotoxin Laboratory at CIGRAS during 2010–2015 in imported commodities, and to determine the level of contamination with AFs and fumonisins in locally grown maize and beans during 2015–2016. Grain samples were analyzed by fluorometry with AflaTest® and FumoniTest® immunoaffinity columns. A total of 3155 samples from imported commodities were analyzed during the 6 year period and included rice (68%), maize (15%), peanuts (11%) and beans (6%). The maximum aflatoxin levels observed corresponded to white maize (420 μg kg^{-1}), yellow maize (410 μg kg^{-1}), and red beans (360 μg kg^{-1}). A total of 104 samples of locally grown maize and beans were collected in farmers fields and markets. Maximum AFs levels corresponded to white maize (180 μg kg^{-1}), yellow maize (110 μg kg^{-1}) and red beans (48 μg kg^{-1}). From the total of 54 maize samples analyzed for fumonisins, 48% had levels ≥ 2 mg kg^{-1}. The highest fumonisin concentration corresponded to white maize, with a maximum level of 12 mg kg^{-1}.

Development of a LC-MS/MS method for Multi-Mycotoxins determination in maize, beans, rice, peanut, and wheat

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Mycotoxins are toxic fungal metabolites commonly found in grains that can cause diseases and death of animals and humans. Therefore, analytical tools for monitoring and preventing these toxins to enter the animal and human food chain are required. The objective of this work was to develop a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for simultaneous detection and quantification of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), ochratoxin A (OTA), fumonisin B_1, and T-2 toxins in maize, beans, rice, peanut, and wheat. To determine possible matrix effects, three levels (5, 10, 20 μg/kg) of each standard were added to the blank matrix. Twenty-five grams of ground samples were blended for 5 min with 100 mL of a methanol: water: formic acid (79:20:1) solution and centrifuged at 3000 rpm for 5 min at 10°C. A 10-mL aliquot of the supernatant was diluted with 10 ml of water and passed through a 1.5 μm glass microfibre filter and a 0.2 μm syringe filter. The final slurry was injected into a HPLC-MS/MS equipped with a C18 column and in positive ionization mode. For all mycotoxins, the best results were obtained using the mobile phase methanol (97%), water (2%) and formic acid (1%) at a flow rate of 400 μL/min with a gradient elution program and a sample injection volume of 5 μL. Linear responses were obtained for all mycotoxins, mean recoveries ranged from 8.5% to 308.9%, limits of detection ranged between 0.8–9.5 μg/kg, and limits of quantification between 2.7–31.6 μg/kg.

Tropical and subtropical disease incidence on ornamental crops in south Florida from 2013–16

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Florida leads the nation in production of tropical foliage, and Miami-Dade County ranks number one in nursery and landscape production with sales reaching $2 billion annually. The University of Florida's Extension Plant Diagnostic Clinic located in Homestead is an important resource for the agricultural community. Data collected from 2013–16 from 2,410 ornamental plant diagnostic samples provided an insight of the major diseases affecting commercial production and landscapes in south Florida. Results included three major groups of different plant hosts type; herbaceous ornamental or indoor plants (59%,) woody plants (15%), and palms (6%). Fungi accounted for 44% and were the most prevalent plant pathogens with the major genera consisting of *Fusarium* sp., *Colletotrichum* sp. and *Rhizoctonia* sp. The most susceptible hosts included various types of mandevilla, 11%; ficus, 2.4%; and dracaena, 2.2%. *Mandevilla splendens* was determined to be the most susceptible ornamental plant species and tested positive for Phytophthora root and stem rot and foliar blight, and Fusarium root and stem rot. In addition, ***M. splendens*** is susceptible to bacterial pathogens including *Xanthomonas, Pseudomonas* and *Ralstonia.* Further studies focusing on disease management are currently underway.

Molecular and morphological identification of *Colletotrichum* species in papaya fruits (*Carica papaya*) 'pococí' hybrid

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Anthracnose, caused by *Colletotrichum* spp, is the main post-harvest disease of papaya worldwide. In this study, 129 *Colletotrichum* monosporic isolates were established in PDA medium from papaya fruits exhibiting symptoms of anthracnose that were collected in three main production areas of Costa Rica. All isolates were characterized by colony morphology and PCR with taxon-specific primers CgInt for *C. gloeosporioides* sensu lato, CaInt2 for *C. acutatum sensu lato*, and with specie-specific primers GmF / GmR for *C. magnum* and CcapF / CapR for *C. capsici*. A multilocus phylogeny approach was used to identify a subset of isolates to species level with sequences from the ribosomal internal transcribed spacer region (ITS) and partial sequences of the actin (ACT), β-tubulin (TUB2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and chitin synthase 1 (CHS) genes. Phylogenetic analyses were performed through Bayesian Inference and Maximum Likelihood. Six species were identified, *C. fructicola*, *C. tropicale*, *C. theobromicola*, all in the *C. gloeosporioides* species complex, *C. simmondsii*, belonging to the *C. acutatum* species complex, *C. capsici* and *C. magnum*, with the latter being the dominant species. To the best of our knowledge, this is the first report of *C. fructicola*, *C. tropicale*, *C. theobromicola* and *C. simmondsii* associated to anthracnose of papaya fruits in Costa Rica.

Machine learning techniques applied to forecast Black Sigatoka disease development rate using meteorological data

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Meteorological data coupled with direct observations of symptom development in the field is a common method used in forecasting black Sigatoka disease (BS) evolution. This methodology is time consuming and depends on the ability of systemic fungicides to arrest infections at early stages of the disease development. Nowadays, techniques are being used in agriculture research to develop models of crop production prediction and disease forecasting. In consequence, machine learning was used to predict the rate of development of BS in banana plantations in the Caribbean of Costa Rica based on meteorological data and the state of evolution of this disease from 2003 to 2015 by means of regression at two experimental plantations with no fungicide applications. Classical techniques (e.g. ARIMA, linear regression, elastic nets regression) and machine learning techniques (e.g. support vector machines, recurrent neural networks, and ensemble methods like bagging and gradient boosting) were probed. The main findings were: 1) The highest R2 was 60%, 2) The highest R2 were reached with linear models like support vector regression with linear kernel, 3) As little as three meteorological variables can be used because of the correlations detected among variables. Currently this research is focused on the extension of these findings to other farms and the extension of the model to be applied in an early warning system, which does not require the regression task.

Effect of microbial amendments and chemical nematicides on root health and nematode populations in banana (*Musa* sp.) plantations

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One of the most limiting factors in banana production worldwide is the damage caused by plant parasitic nematodes that attack the root system, among which *Radopholus similis*, *Pratylenchus coffeae* and *Helicotylenchus milticinctus* are most important, causing root necrosis, uprooting and general loss of vigor. Application of chemical nematicides with a high economic and environmental cost is the main control method, but it results in severe declines of beneficial soil microbial populations. The objective of this study was to compare the effect of two treatments: conventional nematicides (Terbufos and Vydate azul) and the microbial amendment Cronox (*Trichoderma asperellum* and *Pochonia chlamydosporia* in an activated carbon vehicle). The applications were directed to the soil near the succession plant. Variables analyzed were: i) nematode populations; ii) root abundance, quality and distribution; iii) plant

performance in terms of growth, fluorescence and [chlorophyll]. Cronox was as effective as the chemical nematicide in the control of nematodes, and stimulated the differentiation of more roots of higher quality. Plant growth did not differ between treatments; plants treated with Cronox showed higher chlorophyll content (SPAD=41,8 vs 39,3) and improved physiological activity in the field (Fv/Fm= 0,57 vs 0,68). In addition, we show the importance of the standardization of laboratory protocols for nematode population counts.

An unusual occurrence of *Synchytrium* on *Phaseolus vulgaris* and *P. coccineus* in Costa Rica

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In January 1975 an unusual disease of common and cubaces bean (*Phaseolus vulgaris* and *P. coccineus*) was observed in five farms in the province of San Jose, Costa Rica. At a meeting of the Asociación Latinoamericana de Fitopatologia in San Jose, that same year, this disease was named agalla aérea and was reported to be caused by an unknown fungus. The disease was characterized by small galls, 1 to 3 mm diameter on leaves, stems, pods and flower parts. Galls eventually became sori, exposing a golden-yellowish center. Heavily infected common bean plants were often stunted with malformed stems, petioles, leaves or flower parts. Examination of the fields indicated that *P. coccineus* planted adjacent to common bean was the source of inoculum. Growers had previously observed this disease on cubaces beans. A subsequent examination of stored disease samples identified the causal organism as a species of *Synchytrium*, a member of the phylum Chytridiomycota, zoosporic fungi phylogenetically related to the true fungi. Both known and unknown species of *Synchytrium* are reported on *Phaseolus* spp. Since infected plants showed no evidence of resting spores, only sporangia, according to Karling, this species would group in the subgenus Woroninella. This species is similar to a *Synchytrium* found on *P. coccineus* by Standley in 1925 and could be *S. phaseoli* as described by Weston in 1930. Species identification using DNA sequencing is in progress.

Biomolecular identification of *Phytophthora, Pythium* and *Phytopythium* isolated from root and rhizosphere of citrus trees (*Citrus* spp.)

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In the Peruvian coast, microclimates are ideal for the production of citrus trees (*Citrus* spp.), but frequently the roots of the plants are affected by oomycetes reducing annual production. For this reason, the aim of this research was to identify the oomycetes presents using molecular methods. Samples from both secondary roots and rhizosphere were recovered from eight fields corresponding to the four main producing regions along the Peruvian coast (Piura, Lambayeque, Lima and Ica). Oomycetes were isolated on PAR, PARH and V8 agar media. Molecular identification was performed by amplification of 730 - 820 bp of the Internal Transcriptional Spacer (ITS) region of the rDNA using ITS6/ITS4 primers. The amplified fragments were sequenced using Sanger methodology and phylogenetic reconstruction was done with Bayesian analysis, using two million generations. The results formed clusters with the similar species sequences deposited in the GenBank, including, *Phytophthora nicotianae* (root and soil isolate), *Phytophthora parsiana* (root isolate), *Pythium splendens* (root isolate), *Pythium aphanidermatum* (soil isolate), *Pythium ultimun* (soil isolated), *Pythium deliense* (soil isolate), *Phytopythium vexans* (root and soil isolate), *Phytopythium amazonianum* (soil isolate) and *Phytopythium cucurbitacearum* (root and soil isolates). A species related to *Pythium guangxiense* (soil isolate) was also identified. These results will be used for a better plantation management.

Pathogens linked to the decline of *Casuarina equisetifolia* (ironwood) on the Western Pacific tropical island of Guam

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With a landmass of 212 square miles, Guam is the largest island in the Western Pacific geographic region known as Micronesia. *Casuarina equisetifolia* (ironwood) grows nearly everywhere (beaches, landfills, road shoulders, cleared land, and vacant lots), with the exception of undisturbed limestone forests. All Mariana Island landforms support ironwood trees: coral sand, limestone uplands, and volcanic uplands. Though normally considered a healthy and a highly environmental tolerant tree, a reduction in the health of some tree stands was noticed in 2002. Ironwood trees at these sites exhibited classic signs of decline: symptoms were nonspecific (thinning foliage and dieback of small branches) and causality could not be attributed to any single biotic or abiotic factor. In 2008, the condition was designated as ironwood tree decline (IWTD). In 2010, various modeling techniques were applied to survey data collected in 2008 and 2009 from 1,427 trees at 38 sites. Modeling identified levels of landscape management practices and the presence of conks and termites as significant predictive factors. Subsequent research in 2012 determined the conks were primarily those of the pathogenic heart-rot fungus *Ganoderma australe* species complex. Based on seedling inoculations studies in 2013 and tree survey results for *R. solanacearum* using Agdia Inc. specific immunostrips in 2015, it was concluded that the bacterial wilt pathogen *Ralstonia solanacearum* was also associated with IWTD.

Plant Diseases of the Western Pacific tropical island of Guam

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Over 300 plant pathogens, mainly of vegetables and fruit crops, have been reported from the tropical island of Guam. Accounts appear among the many annual reports, abstracts, journal articles, and fact sheets that have been produced since 1905. Efforts are being made to consolidate first reports and supporting articles into a reference document, which will become known as the Index of Plant Diseases in Guam. It is slated to be placed on the University of Guam website in the fall of 2017. The Index is intended as a general reference of plant diseases in Guam and is derived without bias from a wide array of resources with varying degrees of authenticity. Project funding is provided through a 2014 Western Sustainable Agriculture Research and Education Professional Development Program grant EW-14-006: Plant Disease Diagnostic Training for Agriculture Professionals in Guam and the Northern Mariana Islands. Grant funds were also used for a four-day plant diagnostic training held May, 2016. Workshop presenters included Dr. Robert Schlub, Dr. Raghuwinder Singh, Dr. James McConnell, Dr. Andrea Blas, and Mr. Jesse Bamba. Diseases discussed included those that have been known for years to occur on Guam (papaya ring spot, tinangaja, anthracnose, target spot, and banana bunchy top) those that recently emerged (citrus canker and citrus greening) and ones that have yet to be named or identified (tomato with interveinal purpling with chlorosis).

***Sclerotium rolfsii* associated to citrus seedling blight in Chicontepec, Veracruz, Mexico**

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Veracruz State is one of the main citrus producing areas in Mexico. On October 2016, a disease on sour orange rootstocks (*Citrus aurantium*) seedlings was observed on a citrus nursery at Chicontepec, Veracruz. Symptoms consisted of girdling stem lesions near the soil line. Severely infected seedlings turned yellowish, wilted and eventually died. White mycelial mats were observed on the stems of the infected plants. Infected stems sections (1 mm) were surface sterilized with sodium hypochlorite (1%) for 1 min, then rinsed with sterile distilled water, transferred to potato-dextrose-agar (PDA) Petri dishes and incubated at room temperature. A fungal white and cottony colony was consistently isolated and, after 2 wk, globoid dark-brown sclerotia were produced. Colony and sclerotial characteristics corresponded to *Sclerotium rolfsii*. Pathogenicity test were conducted by placing 15 sclerotia near the stem of healthy six-month old *Citrus aurantium* seedlings grown on sterilized substrate (1:1 peat moss + vermiculite). Disease symptoms were observed at 2–3 wk after inoculation, and the pathogen, with the same morphological/cultural characteristics, was re-isolated from infected stems to fulfill Koch's postulates. To our knowledge this is the first report of *Sclerotium rolfsii* on *Citrus aurantium* seedlings in Mexico.

Morphological and molecular characterization of *Fusarium oxysporum* f.sp. *apii* in Costa Rica

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Celery is cultivated in different regions of Costa Rica, including, Escazú, San Cristobal, Alfaro Ruíz, Paraiso, El Guarco and the northern part of Cartago. Total production is approximately 25 metric tons annually. This crop is affected by different pathogens but in the last few years *Fusarium oxysporum* has caused substantial at the plantation level. To better identify and describe the pathogen, isolates were obtained from diseased plants that showed a variety of symptoms including: delayed plant growth, dwarfism, yellow and wilting foliage, as well as blackening and damaged root systems. Koch postulates were done in the greenhouse, and a morphological description of the pathogen was done based on different characteristics of spore quantity and the presence macro and microconidia on carnation leaf agar (CLA), as well as colony appearance (color and pigmentations), which were observed on potato dextrose agar (PDA). Molecular characterization was performed using sequences from EF-1a, Histone 3 and β-tubulin. Results matched to the four isolates found at NCBI related to *F. oxysporum* f.sp. *appi* and based on data from the University of California, Davis, our results further suggested that we are dealing with the presence of race 3 or 4 in Costa Rica.

Etiology of stem rot on *Hylocereus* spp. cause by *Enterobacter hormaechei* in Costa Rica

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In 2011, infected samples were examined to identify the causal agent of stem rot of *Hylocereus* spp. in Costa Rica. During the rainy season, symptoms associated with the disease included a small yellowish rot that typically started from the edge of the cladodium and extended inward towards the stem. Eventually the whole stem decayed. During the dry season, orange circular spots appeared on the stem, turning dark brown. The identification of the causal agent was based on isolation, identification and characterization of biochemical and molecular tests, as well as verification based on Koch's postulates. Results indicated that the symptoms were caused by the same bacteria. According to biochemical tests (Vitek 2), there was a 98% probability of being *Enterobacter cloacae* spp. *cloacae*

(bionnumber 2627634553533010). Based on sequencing of the 16S region, the bacterium showed 99% similarity with *Enterobacter hormaechei* (KJ999997). Both species belong to the complex of *Enterobacter cloacae*. According to Koch's postulates, a similar symptomatology was obtained on plants from the field and on inoculated plants. This is the first report of this pathogen causing damage to the stem of *Hylocereus* spp. in Costa Rica.

Presence of calcium oxalate crystals associated to an abiotic disease known as "Mancha Blanca" on *Hylocereus* spp.

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A survey of the diseases affecting pitahaya (*Hylocereus* spp.) in Costa Rica was carried out during 2012 and 2013. Among the symptoms observed, the most common was a whitish thin layer covering the surface of mature tissues, characteristic of the abiotic disease called "white spot". The origin and composition of this layer is unknown. The presence of calcium oxalate crystals associated with these symptoms was determined by electron microscopy, X-ray spectrometry and X-ray diffraction. Calcium oxalate concentration was 246 times higher in symptomatic cladodes compared to healthy ones. Electron microscopy analysis showed rafidium crystals and cubes, composed mainly by carbon, oxygen and calcium, in symptomatic material. Moreover, the presence of oxalates in diseased tissue was confirmed by X-ray diffraction. This research established for the first time an association between calcium oxalate presence and the "white spot" disease of *Hylocereus* spp. in Costa Rica.

Etiology of stem canker caused by the fungus *Neoscytalidium dimidiatum* on *Hylocereus* spp., in Costa Rica

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A survey of the diseases affecting pitahaya (*Hylocereus* spp.) in Costa Rica was carried out during 2011 and 2012. One of the most important, due to its high incidence, was the stem canker. The symptoms observed were irregular orange and red small spots and beige-gray colorations with raised center. The identification of the causal agent was based on isolation, identification and characterization based on morphology and molecular characteristics, as well as verification based on Koch's postulates. Based on the morphological characteristics and sequencing of the ITS region, the fungus showed a 99% similarity with *Neoscytalidium dimidiatum* (ac.no.KJ513460). According to Koch's postulates, a similar symptomatology was obtained on plants from the field and on inoculated plants. This is the first report of this fungus causing damage to the stem of *Hylocereus* spp. in Costa Rica.

***Herbaspirillum rubrisubalbicans* causing important emergent diseases in sugarcane in Mexico**

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Sugarcane (*Saccharum* spp. hybrid) is one of the most important crops for sugar production worldwide. Since 2014 producers of the Golfo, Sur and Pacific zones have observed symptoms such as mottled stripe with reddish color; later leaves turn dark brown reducing the photosynthetic area for sucrose synthesis in ITV 92-1424. For this reason, the aim of the present study was to identify the causal agent of this symptomatology. Sugarcane leaves from symptomatic plants were disinfested and small pieces were put on papa-dextrose-agar (PDA) and King's B agar media, incubated at 28°C for 8 days. After 72 h, white-cream colonies were developed in King's B medium. In addition, fungal colonies with purplish red color in PDA medium after five days were observed. Both were transferred to obtain pure cultures. Later, 51 bacteria and 14 fungi were subject to DNA extraction and PCR amplification of the 16S rDNA and ITS rDNA region. PCR amplicons were sequenced and analyzed by Bayesian phylogenetic inference. The study revealed that the bacterium *Herbaspirillum rubrisubalbicans* was the causal agent of the mottled stripe and the fungi corresponded to endophytic *Clonostachys rosea*. Pathogenicity test were performed in plants from tissue culture and all bacteria were able to reproduce the diseases when inoculated in healthy plants, while no symptoms were observed in inoculation with *C. rosea*. Furthers studies are in progress to identify sugarcane varieties resistance-related phenotypic data to generate a model to predict the severity of the disease using genotypic information through DArT technology.

Isolation of a new *Rathayibacter* species from *Sporobolus cryptandrus* in Northcentral Idaho

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Members of the genus *Rathayibacter* are vectored by anguinid nematodes in the genera *Anguina* and *Afrina*, and cause bacterial head

blight diseases of grasses. One member, *Rathayibacter toxicus*, is a Select Agent in the United States. It produces extracellular polysaccharides in infected plants resulting in a yellow slime that dries into amber-like droplets on host plant seed heads. *R. toxicus* also produces a corynetoxin that when consumed by grazing animals results in development of a sometimes fatal syndrome known as Annual Ryegrass Toxicity (ARGT). *Rathayibacter rathayi* and *R. agropyri* occur in the US Pacific Northwest (PNW) and produce symptoms in infected plants that easily could be confused with those produced by *R. toxicus*. A survey was conducted in the PNW to determine the presence and range of grasses exhibiting bacterial head blight symptoms and then to isolate and identify the bacteria associated with the symptoms to better understand the occurrence of *Rathayibacter* spp. in the US. Bacterial head blight and seed gall nematodes were found on *Sporobolus cryptandrus*, a common roadside grass in northcentral Idaho, and a *Rathayibacter* spp. was isolated. Based on multilocus sequence analysis of the 16S rRNA gene, recA, gyrB, ppk sequences from this bacterium along with sequences from the seven other described *Rathayibacter* species, this bacterium represents a new unique species. Complete characterization of this putative new species is in progress.

Evaluation of resistance to *Ralstonia solanacearum* in potato germplasm

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Bacterial wilt of potatoes, caused by the *Ralstonia solanacearum* species complex, is a devastating disease that limits production of potato worldwide. The most effective way to control the disease is the cultivation of wilt-resistant varieties. The degree of resistance to *R. solanacearum* (phylotype II, sequevar 1, race 3 biovar 2) was evaluated for 45 accessions of potato wild relatives belonging to the species, *Solanum longiconicum*, *S. woodsonii*, *S. fraxinifolium*, *S. taenotrichum*, *S. canense* and *S. caripense*, collected in Costa Rica, and 29 advanced breeding lines and 11 commercial varieties, conserved as in vitro cultures in the Plant Biotechnology Lab of CIA, University of Costa Rica. Disease incidence of potato genotypes was evaluated weekly for 28 days after inoculation of four-week-old plants with *R. solanacearum* under greenhouse conditions. Resistance to *R. solanacearum* was quantified by wilting index (WI): resistant (0,00-0,25), moderately resistant (0,26-0,50), moderately susceptible (0,51-0,75), and susceptible (0,76-1,00). Two, four, one, and one potato accessions of *S. caripense*, *S. fraxinifolium*, *S. taenotrichum*, and *S. woodsonii*, respectively, were resistant to *R. solanacearum*. One accession of *S. caripense* showed no distinct symptom of bacterial wilt. Three advance breeding lines and one commercial variety were also resistant to *R. solanacearum*. Resistant materials exist for inclusion in our potato breeding program.

Epidemiology of Panama disease (*Fusarium oxysporum* f. sp. *cubense)* on Gros Michel in Costa Rica

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Fusarium wilt or Panama Disease, caused by the fungi, *Fusarium oxysporum f. sp. cubense* (Foc), is one of the most destructive diseases of banana, especially the tropical race RT4 that affects commercial plantations but is not yet present in the Americas. In Costa Rica, Fusarium wilt is a serious problem in small and mid-size plantations that produce the Gros Michel cultivar, often in association with coffee and cacao. The objective of this research was to study the duration in days for the development of Fusarium wilt. Two areas, classified as high or low natural inoculum, were planted with 200 Gros Michel plants. Fusarium wilt was monitored for two years in each area. Results indicated that under conditions of high inoculum, 60% of the plants had first symptoms at 86 days after planting, with 30% presenting stage 2 symptoms and 10% considered in an advanced state. In low inoculum conditions, percentages were similar, however first symptoms were noted at 186 days after planting. No banana bunches were produced in either area, as 100% of plants were dead at seven months in the high inoculum area, while in the low inoculum area 100% plant death was noted at 14 months. These data provide preliminary evidence related to the potential spread of Foc RT4 under small landowner production conditions in Costa Rica.

PCR based methods for the specific detection and identification of *Colletotrichum sansevieriae*

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Sansevieria anthracnose, caused by the newly described species *Colletotrichum sansevieriae*, has become a serious threat to the production and export of *Sansevieria trifasciata*. Accurate and rapid identification methods of this pathogen are pivotal to implement control strategies to prevent its spread to pathogen-free areas. The aim of this work was to develop a polymerase chain reaction (PCR) method for the specific detection of *C. sansevieriae*. Species-specific primers (CstubF/CstubR) were designed from partial β-tubulin gene sequence. These primers amplified a 383 bp fragment of the β-tubulin gene from all *C. sansevieriae* isolates tested but failed to amplify DNA from other Colletotrichum species belonging to *C. gloeosporioides* and *C. acutatum* species complexes and to isolates of *Fusarium oxysporum* frequently recovered from Sansevieria leaves. Furthermore, partial β-tubulin gene amplified using primers T1 and Bt2b and subject to in silico restriction analysis showed a single substitution within a restriction site of MseI (=Tru1I) which was also used in a PCR-RFLP assay that reliably reproduced a two-band restriction pattern specific for *C. sansevieriae*. Both PCR methods were also sensitive enough to detect *C. sansevieriae* strains from naturally and artificially infected Sanseviera leaves without the requirement of isolation for pure cultures.

Toxigenic and non-toxigenic *Aspergillus* spp. associated with dry bean (*Phaseolus vulgaris* L.) in Costa Rica

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Aflatoxins (AFs) are toxic secondary metabolites produced by *Aspergillus* spp. and commonly found as contaminants in grains. Dry beans (*Phaseolus vulgaris* L.), a staple food in Costa Rica, can be subject to high aflatoxin contamination, however, information on the *Aspergillus* spp. found in beans and their capacity to produce aflatoxins is scarce. Therefore, the objective of this work was to isolate and identify *Aspergillus* spp. from bean seeds, and to determine their capacity to produce aflatoxins. Bean samples were collected from three regions in Costa Rica in 2014 and 2015: Northern Pacific, Central and Southern Pacific. A total of 65 *Aspergillus* spp. were isolated from beans, and single spore transfers were made to preserve them in glycerol at –80°C. Isolates were identified using a polyphasic approach. Each isolate was placed on yeast extract sucrose (YES) for aflatoxin quantification by HPLC-MS/MS. *Aspergillus flavus* was the most frequently isolated species (47%), followed by *A. wentii* (14%) and *A. ostianus* (8%). Other *Aspergillus* spp. isolated from beans were *A. parasiticus*, *A. niger*, *A. oryzae*, and *A. foetidus*. Fifty-five percent of the *A. flavus* isolates were aflatoxigenic, and 16% produced aflatoxin B1 in concentrations above 1000 μg kg^{-1}. Most of the *A. flavus* isolates and the most toxigenic ones were found in the northern pacific region. Highly toxigenic *Aspergillus flavus* isolates can be found in beans, therefore, this important staple crop could be an important source of aflatoxins and should be included in monitoring programs in Costa Rica.

Establishment of disease free propagation material for Apio (*Arracacia xanthorrhiza* Bancroft) by tissue culture in Puerto Rico

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Apio (*Arracacia xanthorrhiza* Bancroft) is produced in the highlands of the north-central region of Puerto Rico. This tuber starchy crop is vegetatively propagated and contains the finest starch of all root crops. The disease called “apio corm rot” decreases dramatically the quality and quantity of the farmer’s propagation material on the island, diminishing availability for the next planting season. Currently, propagation material from this area has a germination rate lower than 45%. Plant tissue culture or micropropagation could be used to maintain, grow and increase the availability of disease free propagation material enabling recovery the apio production in this region. There is a lack of studies that describes a complete protocol for apio micropropagation from establishment to hardening. Our research at the Agricultural Experimental Station in Corozal have focused on the development of a protocol for apio micropropagation from establishment, multiplication, rooting and hardening. This poster will describe our advances in the initial phases of the process prior to hardening. We were able to obtain a contamination free establishment phase, a multiplication rate around 1:3 and 95% rooting. While results provide a start for producing enough material for the recovery of production, decontamination protocols need to be tested in order to rescue propagation material from local farmers.

Soybean Cyst Nematode: Potential threat to common bean in Central America

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Common bean (*Phaseolus vulgaris*) is a major food crop in Central America (CA). There are numerous diseases that affect production of common bean. One relatively unknown bean disease is soybean cyst nematode (*Heterodera glycines*; SCN), a soil borne pathogen, first reported in North America in 1954. The cysts are filled with eggs and are readily spread in infested soil on equipment or with seed containing soil particles. Once established in an agricultural area it is almost impossible to prevent the spread of SCN. In the United States, SCN is the most important soybean disease which now threatens common bean production in some major bean growing areas. In South America SCN occurs in Columbia, Brazil and Argentina and substantial losses are reported in Argentina and Brazil. There are no current reports of SCN in CA. However, soybean production in CA has increased over the past 30 years, thus there is enhanced potential for introduction of SCN. Recent research has shown that yields of common bean can be reduced by SCN and there is evidence that SCN will interact with soil borne fungal pathogens to cause greater damage to plants. Fortunately, within the germplasm of *P. vulgaris* there are sources of resistance to SCN. An awareness of the potential introduction of SCN into CA could prevent serious problems with this nematode in the future.