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Bacterial blight, caused by *Burkholderia glumae*, is attacking *Brachiaria* in Colombia: First report

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In 2009, a bacterial blight was observed in a greenhouse at CIAT headquarters, attacking plants of *Brachiaria humidicola*. Because *Brachiaria* grasses are grown as pastures throughout South America, this new disease could, potentially, have significant economic implications. Symptoms included chlorotic streaks, necrosis, and yellowing of flag-leaf margins. Isolates cultured onto King's B medium were cream-colored, producing a yellow, diffusible, non-fluorescent pigment. For molecular identification, the 16S rDNA gene was amplified from all isolates, using primers 27f and 1525R, and obtaining a PCR product of 1500 bp. The nucleotide sequences were 99% identical to those of GenBank accession no. CP009435.1 and other entries of *Burkholderia glumae*. To confirm pathogenicity, 10 plants were inoculated by injection with bacterial suspensions at 10^8 cfu/mL for each of nine genotypes: *B. decumbens* CIAT 606; *B. brizantha* cv. Marandu; *B. brizantha* cv. Toledo; *B. ruziziensis* CIAT 4402; CIAT 36061 cv. Mulato I, CIAT 36062, CIAT 36087 cv. Mulato II, BR02/1752, and BR02/1794. Control plants received sterilized distilled water. All the plants were incubated in micro-chambers, placed in a greenhouse at 28°C, and evaluated 6 days after inoculation. Inoculated plants showed chlorotic streaks, necrotic lesions, and a yellowing flag leaf. Control plants showed no symptoms. This is the first report on *Burkholderia glumae* causing bacterial blight in *Brachiaria* in Colombia.

Sensitivity of Botryosphaeriaceae species causing dieback in blackberry (*Rubus* sp.) to fungicides of different mode of action

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Dieback and stem canker of blackberry (*Rubus* sp.) is commonly found in commercial plots in Michoacán, Mexico. A complex of fungi of the Botryosphaereaceae family causes these symptoms. The objective of this study was to evaluate the *in vitro* sensitivity of *Lastodiplodia theobromae*, *L. pseudotheobromae*, *L. parva*, *Neofusicocum parvum*, *N. ribis*, *Pseudofusicocum stromaticum* and *N. kwanbonambiense* to fungicides from different groups and modes of action in APDA media amended with commercial dose, 50 mg L⁻¹, 5 mg L⁻¹ of the fungicides Difenconazole, Tiabendazole, Thiophanate methyl, Ciprodinil + fludioxonil, iprodione, potassium phosphite and propolis. The experiment was conducted on a lab bench in a completely randomized design with treatments arranged in factorial design (Fungicide dose, species of fungi and media). The experiment was conducted twice. Significant differences ($p < 0.001$) between species fungicides and doses were found. Ciprodinil + fludioxonil, inhibited mycelial growth in all species tested at the three dose evaluated, followed by iprodione, thiophanate methyl and thiabendazole. The fungicides with the lowest effect on mycelial growth were difenoconazole, potassium phosphite and propolis. The latter did not inhibit mycelial growth of any dose tested. Potassium phosphite inhibited 100% of mycelial growth in *L. parva* and *P. stromaticum*, but the rest of the species were inhibited only by 15 to 25% at the commercial dose. The results indicated that there is differential sensitivity of different species Botryosphaeriaceae to fungicides, which must be confirmed *in planta* prior to the recommendation to producers.

Fungi associated with fruit rot of imported rambutan in Texas

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From 2011 to 2013, a survey of fungi associated with fruit rot of imported rambutan was conducted in Amarillo, Texas. A total of 24, 23, 43, and 15 decaying fruit from Guatemala, Hawaii, Mexico, and Thailand, respectively, were sampled. Infected fruit sections (1 mm²) were surface sterilized, rinsed with sterile deionized water, and transferred to PDA amended with ampicillin and rifampicin. Using taxonomic keys based on morphology, fungi were identified to the genus level. Out of 142 fungal isolates recovered from decaying rambutan from Guatemala, the most common genera were *Pestalotiopsis* (30%), *Phomopsis* (20%), *Glioccephalotrichum* (12%), *Beltrania* (11%), *Colletotrichum* (9%), *Xylaria* (3%), *Curvularia* (2%), *Fusarium* (1%), and genera in the *Botryosphaeriaceae* (1%). Out of 98 fungal isolates recovered from decaying rambutan from Hawaii, the most common genera were *Lasmenia* (43%), genera in the *Botryosphaeriaceae* (21%), *Cladosporium* (6%), *Pestalotiopsis* (4%), *Phoma* (4%), *Colletotrichum* (1%), *Curvularia* (1%), and *Guignardia* (1%). Out of 306 fungal isolates recovered from decaying rambutan from Mexico, the most common genera were

Pestalotiopsis (35%), *Phomopsis* (31%), *Colletotrichum* (11%), *Lasmenia* (9%), *Fusarium* (5%), genera in the *Botryosphaeriaceae* (2%), and *Gliocephalotrichum* (1%). Out of 112 fungal isolates recovered from decaying rambutan from Hawaii, the most common genera were *Pestalotiopsis* (35%), *Phomopsis* (31%), *Colletotrichum* (19%), *Guignardia* (5%), genera in the *Botryosphaeriaceae* (2%), *Greeneria* (2%), *Bipolaris* (1%), and *Cladosporium* (1%). Unidentified fungi were 2% (Mexico), 4% (Thailand), 9% (Guatemala), and 18% (Hawaii). Several genera listed, including *Gliocephalotrichum*, *Lasmenia*, and *Fusarium*, have species that have been reported as causal agents of fruit rot, rachis necrosis, flower abortion, leaf spots, vascular wilt, and/or inflorescence wilt of rambutan. The potential exists for fungal populations in fruit of rambutan to be imported or acquired during transport from one geographical location to another, with the possibility that certain fungi may be pathogenic on other crops.

Fungi associated with maguey pulquero (*Agave salmiana* Otto ex Salm-Dyck) in the region of Otumba, State of Mexico, Mexico

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In order to determine the phytosanitary condition of maguey pulquero (*Agave salmiana*) at the State of Mexico; at 2014 a supervision was conducted in growing fields in the region of Otumba and San Martín de las Pirámides. Ten plots of one hectare each one, were selected; in each plot, a survey was conducted in Greek guard for detect symptoms in leaves (pencas) and meristem (cogollo), the main symptoms observed were dry and wet rot; soft rot with yellowish halo; spots with concentric rings, small necrotic spots and meristem rot; of these different symptoms, samples were collected and transported to the National Center of Phytosanitary Reference-Directorate General of Plant Health (DGSV, by its initials in Spanish) for their diagnostic. At the laboratory, pieces of 1 cm² between the transition of healthy-diseased tissue, were cut; the pieces were disinfected in sodium hypochlorite at 1% for 1 min, subsequently washed three times with sterile distilled water and placed in Petri dishes with potato-dextrose-agar medium (PDA), and humid chambers (Petri dishes with humid absorbent paper). The isolates were identified by morphometric keys and scientific literature. According to the diagnostic, the fungi with greater presence were: *Colletotrichum agaves*, *Coniothyrium concentricum*, *Stagonospora gigantea*, *Verticillium* sp., *Fusarium solani*, *Fusarium* sp., *Cladosporium* sp., *Alternaria* sp., *Phoma* sp., *Acremonium* sp., *Sordaria* sp., and *Asterina* sp. The National Service of Agro Alimentary Health, Safety and Quality (SENASICA, by its initials in Spanish) through the DGSV, has the responsibility to detect quarantine pests that threaten plant health for Mexico, in this case the organisms found in *A. salmiana* are not pests subject to quarantine; however, they represent an economic problem for farmers by production losses.

Pathogens of *Eryngium foetidum* L.

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Eryngium foetidum L. commonly known as culantro, Mexican coriander or “recao” is an important herb in daily Puerto Rican cuisine. According to Puerto Rico’s annual gross income, culantro generated \$543,000 with a total of 5,843 ctos. or mazos produced and sold for \$92.93/unit, during fiscal year 2013–2014. Even though culantro is widely used as seasoning, no information is currently available about its pathogens. The objective of our research was to identify fungi and microorganisms associated with symptoms observed in *E. foetidum*. Leaf and root samples showing symptoms were collected from supermarkets, markets, home gardens and farms. Sample sections (4mm) were superficially disinfected and transferred to Potato Dextrose Agar to isolate fungi. Fungal spores were examined using a light microscope for morphological identification. *Colletotrichum* sp., a group of fungi belonging to the Botryosphaeriaceae family and a bacterium, *Xanthomonas campestris* were identified from leaf samples. *Sclerotium rolfsii* was identified from root tissue. This group of fungi was further characterized on the basis of pathogenicity and DNA analysis. The information obtained will be valuable to establish management practices at nurseries around the island.

Sclerotial germination and ascospore formation in *Claviceps gigantea*

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The disease known as “horse’s tooth” in *Zea mays* L. is caused by *Claviceps gigantea* Fuentes et al., an endemic fungal disease mostly in the Toluca Valley and highlands in the state of Mexico. The first report of the disease was by Fuentes y De la Isla (1961), but it was until 1964 when Fuentes et al. (1964) described the causal agent as a new specie in the genus *Claviceps*. Recently, farmers have reported yield losses of 90%. The signs of the disease are under the bracts of the ear as brown sclerotia replacing the grain. This paper describes the time of germination of sclerotia and the development of the sexual bodies to understand its life cycle to facilitate the disease management. Sclerotia were naturally and artificially germinated on whole oats, commercial charcoal, charcoal residues and soil. The results showed highly significant differences ($p \leq 0.0001$) in sclerotial germination when incubated in residual charcoal for three months at 4°C and two additional months at 22–24°C. In this substrate, the germination percentage of sclerotia was 55%, producing an averages of 5 stromatic heads while the other substrates did not induce germination. Stromatic heads were seen 15 d after primordial appearance. In natural field conditions, primordia of stromatic heads produced by sclerotia appeared after six month of incubation at 4°C and 64 d on soil with an average of temperature of 14.07°C. Mature stromatic heads produced a large number of perithecia with thin, large ascospores. Ascospores release was observed 23 d after primordial appearance. The appearance of stromatic heads, their development and maturation in the same sclerotia occurs at different times. The release of ascospores was recorded in video. The DNA was extracted from the sexual structures stipes and capitula.

Fungal diversity in the grains of rice varieties and lines

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In Mexico, rice plantations are located mainly in Campeche State. In this area rice production is affected by several species of pathogenic fungi. The symptoms are stem and leaf spots, and brown discoloration of the grains. Discolored kernels reduced the market value. To help select alternative varieties for the growers, this work records in wetland fields the fungi present in the kernels at harvest of the rice varieties and line: ‘Choca’, ‘Aztecas’, ‘Tres Rios’, ‘Tomatlan’ and ‘Line 1’. Grain surface was disinfested (400 seeds per variety), and placed on moist filter paper at 25°C to allow fungal growth. Based on their morphological characteristics, 13 fungal genera were identified. The main genera present were *Fusarium* and *Curvularia*. The ‘Line 1’ had significantly highest incidences of these two genera (52 and 51%, respectively). This material was followed in order of incidence, by ‘Tres Rios’ (52 and 43%), Tomatlan (49 and 37%), Choca (42 and 42%) and Aztecas (30 and 27%). The variety ‘Choca’ also presented significant incidences (2.4 - 0.3%) of *Aspergillus*, *Verticillium*, *Botrytis* and *Microdochium*. The ‘Line 1’ also presented significant incidences of *Bipolaris* (8.2%) and *Alternaria* (1.9%). The genera *Nigrospora*, *Phoma*, *Cladosporium*, *Penicillium* and *Gonatobotrys* were not significantly present in any of the varieties. The rice variety with significantly less fungi incidence was Aztecas.

First report of *Colletotrichum* sp., associated to the anthracnose in *Oreopanax capitatus* leaves

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The Araliaceae family has about 50 genera and 800 species, distributed in temperate and mainly in tropical areas, being more numerous in the tropics of America and Asia. Among these is bergsalaria (*Oreopanax capitatus* (Jacq) Decne & Planch), this specie has ornamental value as a garden plant and has been growing in botanical collections of public and private gardens; however, its main use is to make flower arrangements with their leaves, and in some parts of Mexico the leaves are used as wrappers for the preparation of tamales. On the other hand, there is a little knowledge about the diseases that affect this specie. During 2015 in Texcoco, State of Mexico, on leaves of *O. capitatus* circular lesions 1–2 mm, dark brown color, and delimited by a yellow halo, were observed; as the symptoms developed, lesions increased in diameter, and became light brown. In severe infections the lesions coalesce and form irregular patches of variable size, in the center of the lesions black colored structures were observed. At the laboratory, pieces of 0.5 cm² between the transition of healthy-diseased tissue, were cut; the pieces were disinfected in sodium hypochlorite at 1.5% for 1 min, subsequently washed three times with sterile distilled water and placed in Petri dishes with potato-dextrose-agar medium (PDA), fungal colonies were purified by hyphal tip. For the identification of structures, cuts were made in the freezing microtome. Isolates and structures, were identified by morphometric keys and scientific literature. From plant tissue cuts, acervuli were identified; on PDA medium, cottony mycelium colonies were developed, initially white color, changing to dark gray at maturity, in the colonies orange pale conidial mass were observed, the conidia were fusiform and unicellular, these morphological characteristics corresponded to *Colletotrichum* sp.

Virus associated to ringspot of wild paddle cactus (*Opuntia* spp.)

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In the States of Morelos and Guerrero, in Mexico, we have observed wild paddle cactus plants with chlorotic ringspots that in many cases cover the entire surface of the cladodes. The causal agent of this disease is unknown. The objective of this study was to determine if a virus is present in cladodes showing chlorotic ringspots. Symptomatic and asymptomatic cladodes were collected from six localities in the southern region of Morelos. Samples were analyzed by scanning electron microscopy to determine the presence of viral particles and light microscopy for inclusion bodies. Additionally, samples were analyzed by RT-PCR using *Tomato spotted wilt virus* (TSWV)-specific primers, *Potyviridae* universal primers, *Potexvirus* universal primers and *Tobamovirus* universal primers. Paracrystalline cytoplasmic inclusions and rod-shaped viral particles were found only in symptomatic cladodes. No cDNA was obtained for TSWV, *Potyviridae* or *Potexvirus*. In contrast, in all 14 symptomatic samples analyzed the expected 400-bp fragment was detected with *Tobamovirus* universal primers designed to amplify part of the coat protein gene. The cDNA obtained from 11 independent samples was sequenced and a similarity search was done in GenBank. Sequence of the virus infecting paddle cactus has 84% similarity with *Rattail cactus necrosis-associated virus* (a *Tobamovirus*). These results suggest that the ringspot of wild paddle cactus is associated with a previously undescribed *Tobamovirus*. Virus purification, whole genome sequencing and mechanical transmission tests to differential plants are in progress to determine the identity of this virus.

Genetic of the resistance to blackleg (*Phytophthora parasitica* Dastur) in roselle (*Hibiscus sabdariffa* L.)

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Blackleg of roselle, caused by *Phytophthora parasitica* Dastur, limits production and reduces yield up to 30%. Symptoms of infected plants are yellowing, necrosis on the crown stem, wilt, and rapid death of the plant. Genetic resistance is a desirable option to control the disease due to host genetic variability, low cost and durability. The aim of this study is to evaluate the genetic resistance to *P. parasitica* in roselle genotypes to be included in a breeding program. To achieve this goal, 52 varieties of roselle were artificially inoculated with the pathogen and seven susceptible varieties and seven resistant were selected. These were intercrossed to determine the genetics of resistance and analyze with DNA markers genetic variation in parental varieties, F1, and F2. Inoculation on the stem crown was done with 5 ml of a suspension of 75×10^3 zoospores mL⁻¹ of pathogen. The varieties were evaluated according to its resistance using a severity index of 0 (resistant) to 5 (susceptible). Tempranilla negra, Tempranilla roja, and UAN-6 showed higher severity while UAN-8, UAN-12, and UAN-25 had lower severity. A diallel cross between 7 varieties identified as resistant and 7 as susceptible was performed, and analysis of P1, P2, F1, and F2, after inoculation will be obtained to calculate genetic effects and inheritance of resistance for use in breeding program.

Spatial Behavior of gladiolus rust in the North region of the State of Mexico

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Gladiolus cultivation, in the northern region of the State of Mexico, is characterized by bringing up its flower production in commemorative dates as Father's Day and All Deaths' day. This region has been considered in the past as a sector with the absence of rust, for that reason we didn't have enough data to prove its presence and we were lack of information about the damages that this disease could cause; but in the last cycles the disease has been presented in these areas. Rust affects the quality of the flower and the corm size, therefore it is considered as a highly important economically disease. The purpose of the current study was to determine the spatial behavior in the gladiolus' rust in the north region of the State of Mexico, in order to elaborate distribution maps. The severity of the plants were estimated during three phenological stages, in 121 georeferenced sampling points, and taking two plots for evaluation during the cycles winter-spring and summer-autumn in the years 2013 and 2014. Results indicated that the disease did not occur during 2013 neither in the summer-autumn period in the year 2014. However during the cycle summer-fall 2014 it was seen with incidences of 69.4% in Jiquipilco, 61.1 in Ixtlahuaca and 75.8 in Jocotitlan, arising from the phenological stage of spathe. The disease revealed an aggregated spatial behavior adjusted to the gaussian and spherical model and visualized through drawn maps.

Detection of *Candidatus Liberibacter solanacearum* in potato leaves and its spatial distribution in Toluca Valley

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Zebra chip is a disease associated with the *Candidatus Liberibacter solanacearum* bacterium (CaLs). This disease is vectored by the psyllid *Bactericera cockerelli*, which is present in the majority of potato-producing areas in the United States, Mexico, Central America and New Zealand. Recently, in the growing area of Toluca Valley, Mexico, the incidence of plants showing chlorosis, purpling of foliage, and the upward rolling of leaves throughout the plant has increased. Therefore, the objective of this study was to detect CaLs and its distribution in the production area using geostatistical techniques. In 2013, two commercial plots in the Toluca Valley were selected for study. A total of 121 points were sampled every 10 m using the method of transects over 100 m in which each plant was georeferenced with a global positioning system. The incidence of the disease was determined based on the symptoms observed in the field, which were confirmed by positive results for CaLs in the laboratory. The molecular detection of CaLs was performed using OA2/OI2c and Lp Frag 1-25F / 427R primers. The disease incidence in plot 1 was 57%, whereas in plot 2, it was 45%. In the first plot, no asymptomatic plants were no detected. However, in the second plot, two of 66 plants were asymptomatic plants, and the bacterium was detected in 11 plants. Sequence alignment was performed with the sequences belonging to the three haplotypes currently recognized. The T/C SNP reported for haplotype A was identified in both plots. The spatial distribution of CaLs showed different types of aggregation, as determined using gaussian and spherical models. These results provide useful information for avoiding the introduction of haplotype A via the transport of seed tubers in areas where it is absent.

Associated pathogens, frequency of isolation and effect of sequential inoculation on symptom expression of pepper wilt

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Pepper wilt significantly reduces yields, inducing farmers to change crops or abandon farmlands 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 pathogens have since been associated with it, 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 and oomycete associated to pepper wilt, a survey from July to November of 2013 and 2014 was conducted in Yurécuaro, Vista Hermosa and Tanhuato municipalities in Michoacán, Mexico. Isolations were done on V8 and potato dextrose agar (PDA) media. From 200 samples processed, 45% were identified as *Fusarium* sp., 30% were *P. capsici*, 10% were *Rhizoctonia solani*, 4% were *Pythium* and 11% to an organism not previously identified associated with pepper wilt. Identification at the molecular level through total DNA extraction using the method of 2% CTAB was conducted. Amplifications of the 16S rDNA region was carried out using ITS5 and NL4. The amplified fragments were verified by horizontal electrophoresis and sequenced using Sanger methodology. The presence of *Fusarium* sp., *Phytophthora capsici*, *Rhizoctonia solani*, *Pythium* sp. and *Plectosporium cucumerina*, was confirmed after the sequences were deposited in the GenBank database. This is the first time that *P. cucumerina* has been identified associated with pepper wilt in Michoacán, Mexico. Subsequently, the pathogenicity tests and sequential inoculation assays with selected isolates of *Phytophthora capsici*, *Fusarium oxysporum* and *P. cucumerina* were conducted and it was confirmed that *P. cucumerina*, *F. oxysporum* and *P. capsici* were pathogenic on Jalapeño and Anaheim peppers.

Pantoea stewartii subsp. *indologenes* is responsible for a new bacterial disease in sugarcane

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Sugarcane (*Saccharum* spp. hybrids) is one of the most important industrial crops in Mexico. However, plants with chlorotic streaks in the leaves have recently been observed in plantations in the southeast of the country. These symptoms frequently appear in the middle of the leaves as fine lines with irregular borders. To identify the causal agent, isolations were made from areas of disease progression in affected leaves. Small leaf portions were disinfested and placed on Petri plates with King B medium and incubated at 28°C. After 72 h, light-yellow colonies were observed and selected for physiological and biochemical characterization. Physiological tests revealed that bacteria were Gram-negative, anaerobic, with growth to 4 and 37°C, Indole production, acid produce from Cellobiose, Maltose and Lactose. PCR amplifications were performed with 8F and 1492R and sequencing with 514F and 800R primer set. A phylogenetic approach employing the 16S rRNA gene was used to reconstruct the evolutionary tree with the maximum parsimony method. Results indicate that these isolates belong to *Pantoea stewartii* subsp. *indologenes* with 99 to 100% of maximum identity. Pathogenicity was confirmed by injecting inoculum with a concentration of 10⁸ CFU/mL into the stems of the sugarcane cvs. CP 72-2086 and MEX 79-431 grown in the greenhouse. All test strains developed fine chlorotic streaks on the young leaves by 8 days after inoculation. None of the un-inoculated control plants developed chlorotic symptoms. Isolates were also infiltrated into tobacco leaves and these showed a hypersensitive response by 4 days. *P. stewartii* subsp. *indologenes* has not previously been reported as affecting sugarcane in Mexico, this result can be considered during the selection of clones to be used as seed in the next planting period.

Is Coconut Lethal Yellowing present in Puerto Rico?

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Coconut Lethal Yellowing (CLY), a phytoplasma disease, is regarded as the most important significant factor affecting coconut worldwide. This disease strikes palms causing leaf chlorosis and inflorescence and fruit's necrosis. In a tropical island such as Puerto Rico, important findings such as: 1) Detection of *Haplaxius crudus*, and 2) Previously detection of 16SrIV phytoplasma in a unidentified palm and in *Cedusa inflata*, a native derbid vector, have increased concerns about its devastating impacts on endemic ecosystems and landscape. In this research the objectives were to detect CLY in palms and in its potential insect vectors in Puerto Rico. Island was divided in three transects: Northern, Central and Southern and symptomatic and asymptomatic palms were sampled. Besides *Cocos nucifera*, other palms species showing symptomatology similar to CLY were sampled. These were: *Gaussia attendata*, *Pseudophoenix sargentii*, *Roystonea borinquena* and *Washingtonia robusta*. Common potential insect vectors were sweep-collected near palms and weeds around them. Palms' DNA was extracted using Fast DNA SPIN Kit for Soil. Insect DNA extraction was carried out using DNeasy Blood and Tissue Kit. Phytoplasmas' DNA was amplified by direct PCR using universal primers P1/P7 for 16S rDNA region. Secondly, nested PCR was conducted using primers LY16Sf2/LY16-23Sr2, and fU5/rU3. Only two samples from *R. borinquena* were positive for phytoplasma corresponding to PPWB phytoplasma, using primers fU5/RU3 with only 16S rDNA region. DNA sequences obtained showed 97% of homology with PPWB belonging to phytoplasma group 16SrIX previously detected in Puerto Rico (Gen Bank KJ817879). Positive amplification of 0.9kb was obtained with *H. crudus* samples using the primers FU5/RU3. These findings indicate that *H. crudus* may act as potential phytoplasma vectors. Thus, CLY phytoplasma is not present in symptomatic palms sampled around the island of Puerto Rico.