

Abstract

The Puerto Rico Plant Diagnostic Clinic (PRPDC) is an important resource for farmers, agribusiness clientele, landscapers and home gardeners. During 2021, disease and pest identification was conducted in more than 1,000 samples. The seed industry in Puerto Rico also relies on the PRPDC for seed analysis of quarantine pathogens for different countries where the seeds are exported. The majority of the samples received are for disease diagnostics. During 2021 a total of 374 samples identifications were shared with the National Plant Diagnostic Network (NPDN). Of importance, *Erwinia chrysanthemi* continues to be an emergent pathogen in corn. A new disease in greenhouses was identified as *Rhizoctonia* aerial blight in *Bougainvillea*, an important ornamental exported in plantings. Powdery mildew has become an emergent problem for mangoes, cucurbit crops, and tomatoes due to conducive environmental conditions. In soybeans, *Phytophthora sojae* was first diagnosed in soybeans. In landscape areas *Phytophthora palmivora* has been identified in canary palms. Citrus nurseries producers have access for testing systemic pathogens at the PRPDC, allowing to market healthy citrus plants. This is in collaboration with the Citrus Clean Plant Network. The PRPDC is a mechanism to detect early diseases, thus preventing epidemics and dissemination by accurate identification. The PRPDC is a way to reach growers and provide disease and pest management recommendations which impact agricultural production in Puerto Rico.

Introduction

In Puerto Rico there are 8,230 farms, averaging 60 acres each (1). Plant disease outbreaks in farms in Puerto Rico cause significant losses in cash crops, fruits, and ornamentals, representing a near 50% decline in crop production. Major crops such as coffee and vegetable production in the southern area faced ongoing outbreaks of fungal, bacterial, and viral diseases. Rapid diagnosis has prevented the geographic expansion of several diseases in the Island.

Objectives

1. To provide an accurate and rapid diagnosis to ensure a better opportunity for the grower to manage pests and diseases.
2. Process diseased samples using traditional and molecular diagnostic tools for samples in 2021.
3. To continue being part of the certification process for quarantine diseases in corn, soybeans, sunflower, cotton and pulses for the winter nurseries in Puerto Rico.

Materials and Methods

- Visual examination of symptoms, isolations on artificial media and enzyme-linked immunosorbent assay (ELISA), ImmunoStrips (AGDIA, Inc., Indiana, USA; AGDEN, USA).
- Bacterial diseases are identified by isolations on selective media (3) polymerase chain reaction (PCR), and pathogenicity tests.
- Fungal diseases are isolated on potato dextrose agar media (PDA) followed by morphological and molecular characterization (Fig. 1).
- Viral diseases are identified with the enzyme-linked immunosorbent assay (ELISA), PCR and RT-PCR and conventional PCR (4).

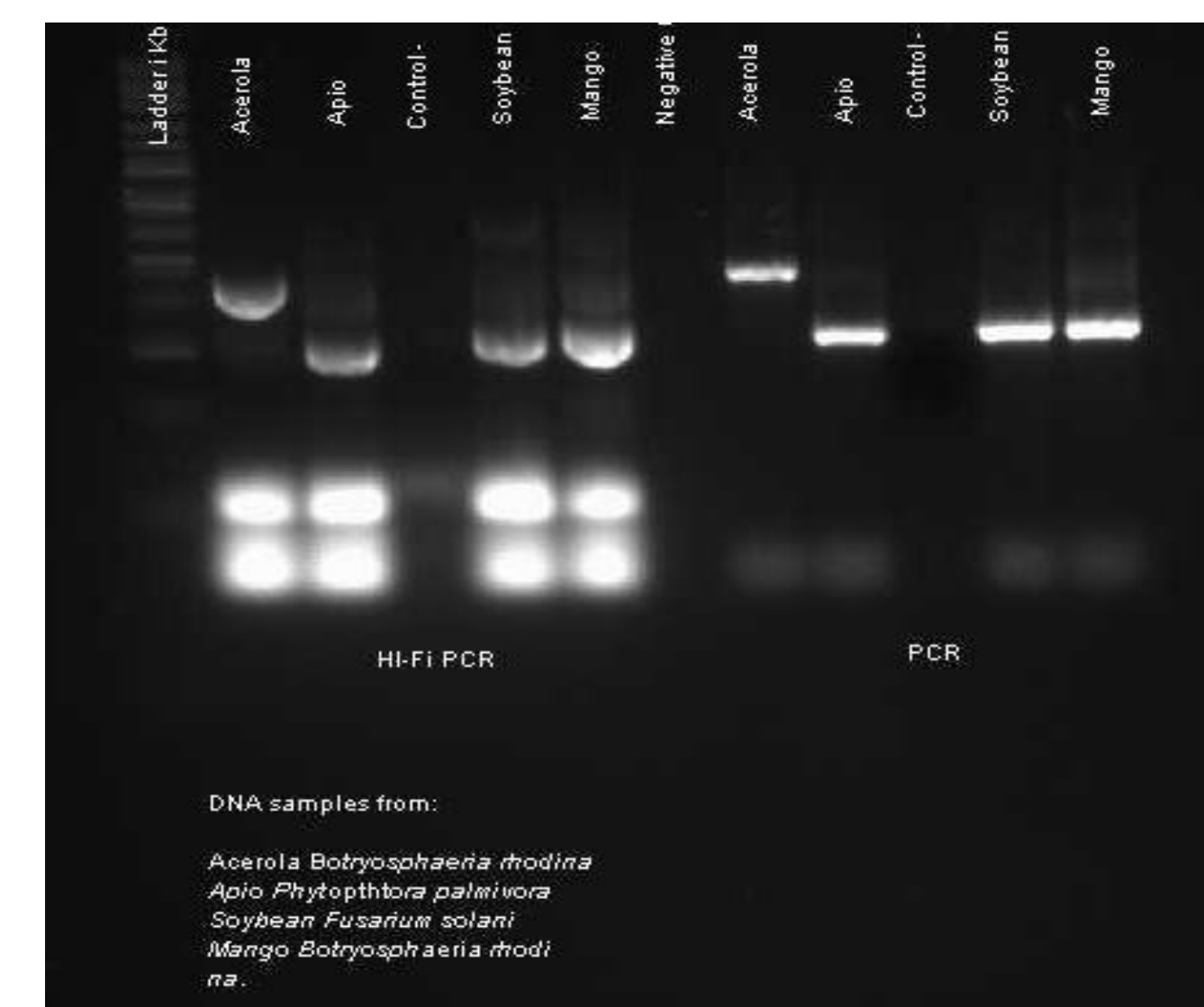


Figure 1. Agarose gel (1%) with amplicons using primers ITS1 and ITS4, with Hi-Fidelity PRC and conventional PCR for: *Botryosphaeria rhodina*, *Phytophthora palmivora* and *Fusarium solani*.

Figure 3. Bougainvillea cuttings and leaves under propagation affected by *Rhizoctonia* blight.

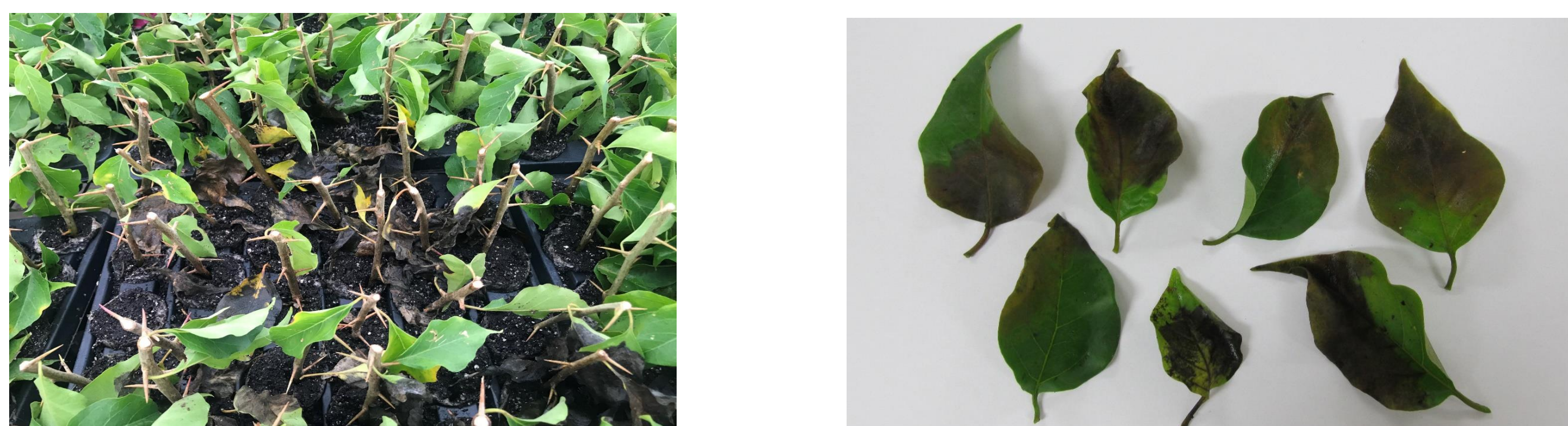
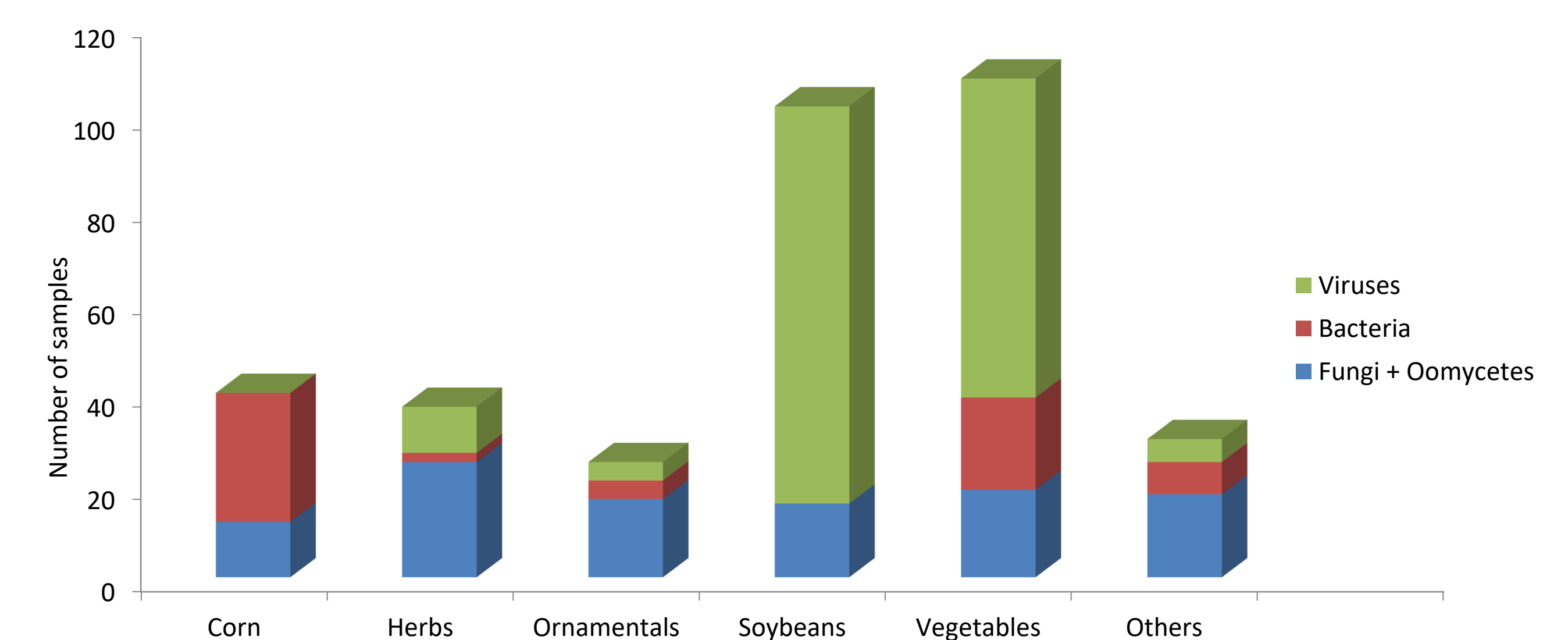


Figure 2. Pathogen distribution of samples submitted in 2021



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Rhizoctonia blight. *Rhizoctonia solani*. Kühn (teleomorph = *Thanatephorus cucumeris* Donk). The disease was very destructive in cuttings growing under greenhouse conditions. Initial symptoms were yellowing of leaves that progressed to water soaked-dark lesions. Mycelia was visible in the developing foliage. Morphological and cultural characteristics were consistent with *Rhizoctonia solani*. Pathogenicity tests were conducted in new cuttings under similar conditions and cuttings developed similar symptoms.

Phytophthora root rot: *Phytophthora sojae* (Kaufman & Gerdman). It was first observed in mature plants growing in promix under greenhouse conditions. Soybeans had root and stem rot, stems had cracks and the cortex and the vascular tissue were necrotic. The pathogen was isolated on PDA media and on V8 agar media non-papillated sporangia were produced. Sporangia were not deciduous in water, oviform to ellipsoidal. Sporangia were 18.7 to 66.0 × 14.2 to 39.1 μm.

Phytophthora but rot: *Phytophthora palmivora* (E. J. Butler) E. J. Butler. Canary Island date palm (*Phoenix canariensis* hort. ex Chabaud) is widely grown in landscapes and home gardens. A dark brown rot from the petiole base of the spear to the adjacent youngest leaves was observed. The palms were killed. Isolations on PDA resulted in an oomycete, and on V8 juice agar media elliptical to ovoid papillated sporangia (30 to 78 × 21 to 39 μm) were consistent with *P. palmivora*.

Bacteria stalk rot: *Erwinia chrysanthemi* Burkholder, McFadden and Dimock. Bacterial stalk rot is observed with frequency during the warm and humid months of June and July in the southern production area. Symptoms were soft rot and darkening of the tissues affecting the stalk and the top of the plant. Lesions progressed from the top to below nodes, leaf sheaths and blades, and rotten tissues produced an unpleasant odor. Cultural and biochemical identification was consistent with *E. chrysanthemi*. Pathogenicity tests were conducted by inoculating 25-day-old maize plants using a syringe with 10⁵ CFU ml⁻¹ bacterial cells in the leaf collar. Symptoms reproduced in 3-5 days after inoculation.

Results

The Plant Diagnostic Clinic in Juana Diaz and the Agricultural Extension Service Clinic (AESC) have processed more than 1,072 diseased samples of 30 different crops and ornamentals. For each sample or query, a report was prepared with management recommendations, emphasizing IPM practices. The economic impact of rapid and accurate diagnostics has minimized the sprays and saved money from applying unnecessary pesticides due to the recommendations provided by the extension service and disease clinic personnel. This was possible because of the use of standard, serological and molecular assays to identify the pathogens that cause diseases and pest identification in the plant pathology and entomology laboratories at the disease clinic. A total of 394 serological tests were conducted for 15 different bacterial and viral pathogens at the PRPDC in Juana Diaz. A total of 345 diagnostic data of the Juana Diaz Clinic Diagnostic and 16 first disease reports were entered in the National Plant Diagnostic Network (NPDN) database.

References

1. Anonymous, 2020. Departamento de Agricultura de Puerto Rico. Resultados del Censo de Agricultura de 2018. ACH17-14.
2. Brown, J. A., M. Idris, I. Torres-Jerez, G. K. Banks, and S. D. Wyatt. 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. Arch Virol (2001) 146: 1581–1598.
3. Schaad, N. W., J. B. Jones, and W. Chun. 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. Third Edition. APS PRESS. pp. 151-166.
4. Wyatt, S.D. & J.K. Brown. 1996. Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. Phytopathology 86:1288-1293. doi.org/10.1094/Phyto-86-1288.

Acknowledgements

- Funding for the Plant and Insect Clinic in Puerto Rico is provided by USDA award # 2021-37621-35791.
- The authors acknowledge Dr. Scott Adkins, USDA/ARS, Dr. Judith Brown, School of Plant Sciences, Department of Entomology, UA Plant Virus Diagnostics Laboratory, and Carrie L. Harmon, Plant Diagnostic Center, University of Florida, Plant Diagnostic Center for collaborating with protocols and methods.