COCONUT LETHAL YELLOWING DISEASE IN CUBA: IDENTIFICATION OF ASSOCIATED PHYTOPLASMAS
Update of the phytoplasma presence

■ THE PROBLEM ADDRESSED

The history of coconut lethal yellowing in Cuba

Among the more serious phytoplasma diseases are lethal yellowing of palms (Eziashi and Omamor, 2010). In Cuba, coconut is used as ornamental plant and some small commercial plantations are present in Baracoa (Guantánamo province), Niquero y Pilón (Granma province) and some municipalities from the central region (Cueto, 1986). In previous report the Cuban lethal yellowing associated phytoplasmas were classified in the 16SrIV group (Llauger *et al.*, 2002). Nowadays there is a National Program to recover the Cuban coconut industry and in this frame it was very relevant to verify the identity of phytoplasmas associated with lethal yellowing to support the further development of strategies to more effectively monitor and manage the disease in the country.

• Symptomatology observed in the coconut trees: yellow leaves in horizontal position and necrotic inflorescences.

■ LATEST RESEARCH RESULTS

The phytoplasma detected in coconut

Coconut lethal yellowing is the most important disease presently affecting the coconut production worldwide. Symptomatic and asymptomatic coconut plants were sampled in some selected areas to verify the identity of phytoplasmas present in Cuba. Diverse phytoplasma ribosomal groups were detected in the samples from symptomatic palms such as 16SrXII, 16SrVII, and 16SrI. In several other palms 16SrIV-A subgroup phytoplasmas were identified. In the *groel* gene the only positive plant from Pilón resulted infected with a phytoplasma that is diverse from the others and identical to the 16SrIV-A strains detected in Jamaica infected coconut palms. The project survey allows the first record of occurrence of 16SrI, -VII and -XII groups in coconut palms in Cuba.

• Locations in Cuba where the coconut plants were sampled.

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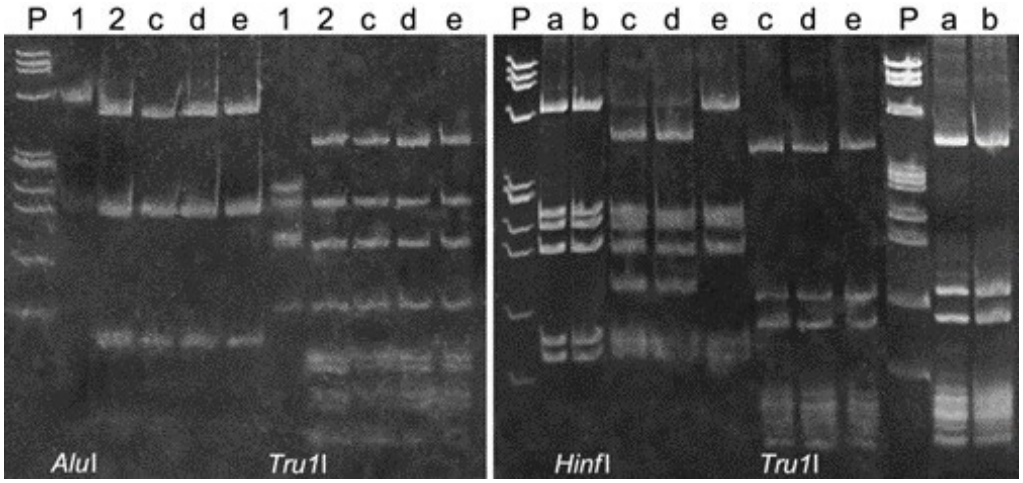
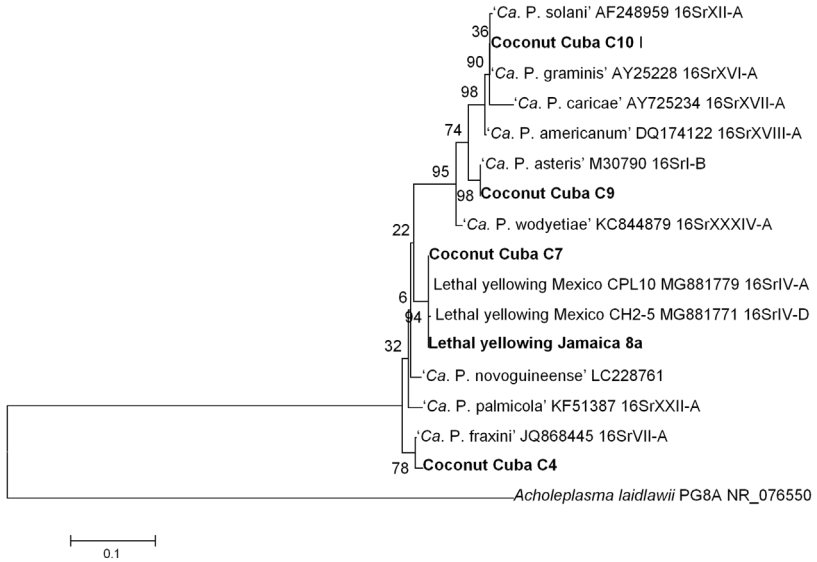
■ THE TROPICSAFE RESEARCH AND DEVELOPMENT ACTIVITY

Molecular identification of the coconut phytoplasmas

Symptomatic and asymptomatic coconut samples were collected from the most active disease *foci* located mainly in the north of the western and central region of the country. A sample from the south-eastern part of Cuba was also included. DNA extraction was performed from 1 g of trunk boring, using CTAB and/or phenol-chloroform based methods. Positive controls from lethal yellowing (16SrIV-A) infected palms from Mexico and Jamaica and 16SrIV-D from Mexico were used. PCR amplification was performed on 16S rRNA gene with universal primers, followed by nested PCR with general and 16SrIV group specific primers and RFLP and /or sequencing. Moreover, the samples positive for 16SrIV were also amplified on the *groel* gene with primers groELF1/R1 in direct and groELF2/R2 (Myrie *et al.*, 2011) in nested PCR. RFLP analyses with *Tru*1I and *Alu*I on 16S rRNA gene amplicons was performed for phytoplasma identification. *Hinf*I enzyme was employed to digest the groELF2/R2 amplicons. Eight out of 16 trunk borings were positive for phytoplasmas and the sequencing confirmed the identity of the detected phytoplasmas as verified with the RFLP analyses.





• Top figure: polyacrylamide gel visualized under ultraviolet light after ethidium bromide staining of RFLP profiles of amplicons obtained from coconut samples with the enzymes listed at the bottom. Left, primers 16S503f/LY16Sr; right, primers groELF2/R2. Samples from Cuba: c, C7, d, C13 and e, C168; samples from Mexico 1, 16SrIV-D and 2, 16SrIV-A; samples from Jamaica a and b; P, marker phiX174 DNA digested with *Hae*III. Bottom figure: phylogenetic tree obtained using Neighbour-Joining method conducted in MEGA6 showing the different phytoplasmas detected in palms in Cuba and in Jamaica in bold.

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■ SCIENTIFIC DATA AND FIRST RESULTS

Molecular diversity of phytoplasmas detected in coconut palms

The results indicate the presence of different phytoplasma ribosomal groups in palms. The 16SrIV strains detected were enclosed in the subgroup –A, while the RFLP on *groel* gene, not amplifying the phytoplasmas enclosed in the 16SrIV-D subgroup, showed that the phytoplasmas in one of the samples from Cuba is identical to the strains from Jamaica. In agreement with previous studies the predominant phytoplasma group detected was 16SrIV. However, strains in 16SrI, -VII and -XII groups were identified by RFLP analyses on 16S ribosomal gene and resulted clustering with phytoplasmas enclosed in these groups. These results indicated for the first time the occurrence of these phytoplasma groups in coconut palms affected with lethal yellowing in Cuba, some of them were already reported in coconut palms in other infected areas (Contaldo *et al.*, 2019).

Sample	Location/ Province	Phytoplasma ribosomal group
C4	Moronta, Camagüey	16SrVII
C6	La Gloria, Camagüey	16SrIV, 16SrXII
C7	Sola, Camagüey	16SrIV
C8	St. Lucia, Camagüey	16SrIV
C9		16SrI
C10		16SrXII
C13	Mayabeque	16SrIV
C168	Pilón Granma	16SrIV

• Results of the survey for phytoplasma detection.

KEY WORDS

Coconut, lethal yellowing disease, phytoplasmas, molecular identification

FURTHER INFORMATION

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AUTHORSHIP

Camilo Paredes-Tomas Research Institute of Tropical Fruit Crops, La Habana, Cuba fitopatologia1@iift.cu
Carlos Oropeza Salin Centro De Investigación Científica de Yucatán, Mexico cos@cicy.mx
Maria Narvaez Centro De Investigación Científica de Yucatán, Mexico maryn@cicy.mx
Wayne Myrie Coconut Industry Board, Kingston, Jamaica waynemyrie@hotmail.com
Assunta Bertaccini *Alma Mater Studiorum* - University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy assunta.bertaccini@unibo.it
Martiza Luis-Pantoja Research Institute of Tropical Fruit Crops, La Habana, Cuba maritzaluispantoja@gmail.com

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