Occurrence of a 16SrIV Group Phytoplasma not Previously Associated with Palm Species in Yucatan, Mexico

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Abstract

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The occurrence of 16SrIV group phytoplasmas in palm species *Sabal mexicana* and *Pseudophoenix sargentii* is reported here for the first time. Palm trees showed leaf decay and leaf yellowing syndromes, respectively. An amplification product (1.4 kb) was obtained in symptomatic *S. mexicana* (18 of 21) and symptomatic *P. sargentii* (1 of 1) palm trees sampled in different locations in Yucatan State, Mexico; five of the positive *S. mexicana* and the positive *P. sargentii* trees died. The identity of the phytoplasmas from these species was determined by restriction fragment length polymorphism profiling with restriction enzymes *Alu*I and *Hin*fI, showing there could be two phytoplasma strains of the 16SrIV group. In one *S. mexicana* palm, the profile was the same as observed with these enzymes for phytoplasmas of 16SrIV-A subgroup, previously associated with *Cocos nucifera* palm trees and, in the rest of the trees, including the *P. sargentii* palm, the profile was for phytoplasmas of the 16SrIV-D subgroup. These identities were supported by analyses of the amplicons obtained by nested polymerase chain reaction by nucleotide-nucleotide BLAST analysis. Geographical distribution of the association *S. mexicana*/16SrIV group phytoplasmas was found widely dispersed in Yucatan State. A potential role of *S. mexicana* palm trees as a permanent source of phytoplasma inoculum is suggested. In addition to *P. sargentii*, other palm species (*Thrinax radiata* and *C. nucifera*) coexisting with *S. mexicana* trees were also sampled and analyzed.

Phytoplasmas are plant-pathogenic bacteria of the class *Mollicutes* that inhabit plant phloem and insects. They are associated with diseases of several hundred plant species (11,15,16,18). They are poorly characterized because they are nonculturable and difficult to isolate (2,28), and characterization relies primarily on molecular methods. Hence, using polymerase chain reaction (PCR) assays, with primer pairs derived from 16S rDNA sequences and restriction fragment length polymorphism (RFLP) analysis and sequencing of the conserved 16S *rRNA* gene (15,16), they have been classified in a monophyletic clade consisting of 18 groups and more than 40 subgroups (27). The '*Candidatus* Phytoplasma' genus has been adopted for a formal classification of these bacteria (13).

The lethal yellowing (LY) disease that affects several palm species is associated with phytoplasmas classified within one of these groups, the 16SrIV group (19), comprising six subgroups: (i) 16SrIV-A, that includes the coconut LY phytoplasma '*Ca*. Phytoplasma palmae' associated with most of the palm species affected by LY; (ii) 16SrIV-B, that includes a phytoplasma found in coconut in Tabasco near the Yucatan Peninsula; (iii) 16SrIV-C, that includes Tanzanian coconut LY phytoplasmas; (iv) 16SrIV-D, that includes *Carludovica palmata* phytoplasma (CPY) (3) and *Phoenix canariensis*, *P. dactylifera*, *P. sylvestris*, and *Syagrus romanzoffiana* with Texas phoenix decline (TPD) phytoplasma (10,11); (v) 16SrIV-E subgroup, that includes phytoplasmas identified in coconuts in Dominican Republic (17) and '*Ca.* Phytoplasma castanae', associated with chestnut witches' broom disease in Korea (14); and, finally, (vi) 16SrIV-F, a novel subgroup that includes a phytoplasma strain discovered in *Washingtonia robusta* palm (8).

Sabal mexicana is a palm species that grows on the coastal plain of the Gulf of Mexico, from the southern part of the State of Tamaulipas to the Yucatan Peninsula (22,23). This palm is called "guano" by Mayan people, who use it in many ways; for instance, the tree is used as an ornamental plant in urban landscape, the trunk is used for rural construction, the leaves are used for house roofs, and different parts are used for handicrafts. Therefore, it is the main income source of many families and economically important in Mexico (1,22). *S. mexicana* is a species reported as resistant to LY by McCoy et al. (19) and still there are no reports in the literature associating it with any 16SrIV group phytoplasmas. However, in 2004–2005, three *S. mexicana* palm trees in the Botanical Garden of Centro de Investigación Científica de Yucatán (CICY), in Yucatán, México, developed leaf decay symptoms and died soon after. Preliminary analysis of DNA from these palm trees using nested PCR, with primers specific for LY group phytoplasmas (10), resulted in positive detection. More *S. mexicana* palm trees have since acquired similar symptoms in CICY's premises and other sites in Yucatán.

Therefore, the purpose of the present study was to determine whether the symptoms in *S. mexicana* in CICY's botanical garden were caused by a 16SrIV group phytoplasma and, if so, characterize it, and determine whether the occurrence of this event was extended to other locations in Yucatan State and whether it had spread to palm trees of other species co-existing with infected *S. mexicana* palm trees.

Materials and Methods

Sampling and evaluation of palm trees. Tissue samples were collected from both *S. mexicana* palm trees showing pronounced leaf discoloration symptoms and from symptomless, presumably healthy palm trees. Trees were sampled by removing either portions of the newest, as-yet-unfurled (spear) leaf or shavings from the interior basal stem using a portable electric drill as previously described (9). Samples were obtained during the course of surveys at four sites in Yucatan State in which affected palm trees were evident during 2004 to 2008. They included the Botanical Garden at CICY in Merida, Periferico urban landscapes in Merida (approximately 10 km from CICY), Chicxulub Puerto (40 km north of Merida), and Ticul (100 km south of Merida). Basal stem samples

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were removed from coconut (*Cocos nucifera*), green thatch palm (*Thrinax radiata*), and Buccaneer palm (*Pseudophoenix sargentii*), with or without foliar yellowing symptoms, that were growing in close proximity to symptomatic *S. mexicana* trees. In order to monitor symptom progression on *S. mexicana*, sites were revisited on a monthly basis for 18 months to collect data on numbers of green leaves, fruit, and inflorescence present on symptomatic and symptomless trees. The data were analyzed using a *t* student test to evaluate the statistical significance between treatments (95% confidence interval, $\alpha = 0.05$).

DNA extraction and PCR assay. Samples (3 g) of leaf or stem tissues were ground separately to a fine powder in liquid nitrogen using a mortar and pestle. Nucleic acids were then extracted from the pulverized tissues by the cetyltrimethylammonium bromide method of Doyle and Doyle (5). Aliquots of each final preparation resuspended in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8) were electrophoresed through standard 1% agarose gels using Tris-acetate-EDTA (TAE; 40 mM Tris-acetate, 1 mM EDTA) as running buffer. DNA in gels was stained with ethidium bromide (EtBr), then visualized by UV transillumination and photographed.

DNA samples were each diluted 1:10, 1:100 and 1:1000 with sterile deionized water and 5 µl of each dilution was used as template in a nested PCR assay following a previously described protocol (10). Briefly, initial amplifications (35 cycles) were performed in 50-µl reaction mixtures employing phytoplasma universal rRNA operon primer pairs P1 (4) and P7 (24). Resulting products were diluted 1:20 or 1:40 with sterile deionized water, and 5 µl of each dilution was then reamplified (35 cycles) with 16SrIV group-specific primer pair LY16Sf (5′-CATGCAAGT CGAACGGAAATC-3′) and LY16Sr (5′-GCTTACGCAGTTAGG CTGTC-3′) (10). Aliquots (10 µl) of final reaction products were electrophoresed through 1% agarose gels in TAE buffer and visualized as described above.

Analysis of PCR products. Aliquots of nested PCR products (5 µl) were subjected to separate digestion with *Alu*I, *Hha*I, or *Hin*fI restriction endonucleases at 37°C for a minimum of 16 h. Products of digests were separated by electrophoresis through 8% nondenaturing polyacrylamide gels using Tris-borate-EDTA (90 mM Tris-borate, 2 mM EDTA) as running buffer. DNA fragment profiles in gels were stained with EtBr and recorded as described above.

Sequencing and phylogenetic analysis of cloned rDNA products. Residual nested PCR products purified from 0.7% agarose gels using a DNA Gel extraction kit (Millipore Corp., Bedford, MA) were ligated with vector pGEM-T (Promega Corp., Madison, WI) and propagated in *Escherichia coli* XL1 Blue cells (Stratagene, La Jolla, CA) according to the manufacturer's instructions. Recombinant plasmids were isolated from individual clones using a Plasmid Midiprep kit (Qiagen, Hilden, Germany). Cloned inserts were sequenced in full by a commercial service (Davis Sequencing, Inc., Davis, CA). To minimize potential sequencing errors, each insert was sequenced two or three times.

A database search of homologous sequences was performed by Blast analysis at the National Center for Biotechnology Information (NCBI) website (http://ncbi.nlm.nih.gov/BLAST). Phylogenetic interrelationships among palm-associated phytoplasmas, representatives of other phytoplasma groups (12), and *Acholeplasma palmae* were assessed based on partial 16S rRNA gene sequences (1,308 bp). Sequences were aligned using ClustalW (26). A neighbor-joining tree was constructed from the aligned sequences using MEGA 4.0.1 software (25). *A. palmae* was used as the outgroup to root the tree.

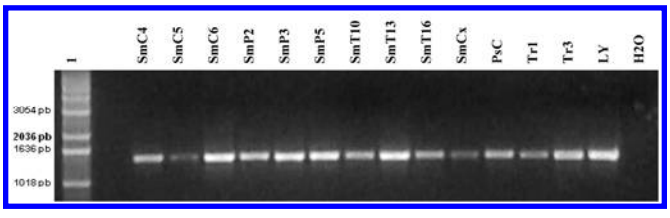

Fig. 1. Amplification of phytoplasma 16S rDNA from *Sabal mexicana* (Sm), *Pseudophoenix sargentii* (Ps), *Thrinax radiata* (Tr), and *Cocos nucifera* (Cn) by a nested polymerase chain reaction assay employing phytoplasma universal rRNA operon primer pair P1/P7 followed by LY16Sf/LY16Sr. H_2O = negative control; 1 = 1-kb DNA ladder.

Results

Phytoplasma detection. The nested PCR assay yielded a 1.4-kb fragment of phytoplasma 16S rDNA from a total of 18 of 21 (85.7%) diseased *S. mexicana* trees assayed from different locations in Yucatan State (Fig. 1). In the Botanical Garden at CICY, two mature bearing *S. mexicana* trees with foliar decay symptoms were first observed in 2004 and a third developed similar symptoms during late 2005. All three palm trees tested phytoplasma positive and eventually died. An additional 18 bearing palm trees that included 5 with and 13 without symptoms were subsequently sampled at this site during 2006 and 2007. Phytoplasma infection of all 5 symptomatic trees was confirmed by nested PCR assay and 1 of these eventually died whereas all 13 symptomless trees produced negative results and have remained alive. Similarly, at Ticul, three of six (50%) mature *S. mexicana* trees with symptoms were found to contain phytoplasmas and one has since died whereas phytoplasmas were undetectable in all eight mature palm trees lacking symptoms. In Chicxulub, only one mature palm with symptoms was observed during 2008. Although this palm tested positive for phytoplasma infection, it did not decline and die during the 3 years of the course of this study. In Periferico, Mérida, where 17 young, nonbearing trees were assayed for phytoplasma infection during 2006 and 2007, phytoplasmas were found in all 6 palm trees exhibiting prominent foliar discoloration symptoms, although none had died by the end of this study. Phytoplasmas remained undetected in the other 11 young palm trees that were symptomless and that did not develop symptoms during the following 2 years.

Other palm species that were co-existing with *S. mexicana* were also studied. In the Botanical Garden at CICY, a symptomatic *P. sargentii* palm with leaf yellowing symptoms was observed during 2006; it tested positive for phytoplasmas and died, whereas five symptomless *P. sargentii* palm trees at this location produced uniformly negative results when analyzed by nested PCR. Furthermore, at this same location, two *T. radiata* palm trees with foliar yellowing proved to be phytoplasma positive, one of which subsequently died (Fig. 1), whereas all seven additional symptomless *T. radiata* trees that were examined at this location produced uniformly negative results.

In Chicxulub, three of seven (42.8%) *T. radiata* palm trees with leaf yellowing symptoms were found to be PCR positive for phytoplasmas and one of these trees subsequently died. Of the four *C. nucifera* palm trees found with foliar yellowing symptoms (three at Ticul and one at Chicxulub), only one palm at Ticul was phytoplasma positive although all four trees eventually died. The solitary diseased palm observed at Chicxulub was phytoplasma positive, too, and died. All seven *C. nucifera* without symptoms sampled at CICY for comparative purposes proved to be phytoplasma negative. Results of palm species evaluations for phytoplasma infection are summarized in Table 1.

Phytoplasma identification and characterization. Restriction fragment patterns generated by *Hha*I digestion of 16S rDNA products amplified by nested PCR revealed no apparent differences among phytoplasmas detected in *S. mexicana* or in *P. sargentii*, *T. radiata*, and *C. nucifera*. (Fig. 2). However, *Alu*I or *Hin*fI digests each revealed two distinct fragment profiles among palm trees. One profile was associated with 14 of 15 *S. mexicana* palm trees, a *P. sargentii* palm, and one of two *T. radiata* trees while a second profile was associated with *C. nucifera* and one *T. radiata* palm sampled at CICY as well as one *S. mexicana* palm sampled at Chicxulub (Fig. 2).

Of the 16S rDNA sequences derived by nested PCR from 13 diseased palm trees, 12 from *S. mexicana* (6 from CICY, 3 from Periferico, and 3 from Ticul) and one from *P. sargentii* were all virtually identical, differing by one or two bases only. Pairwise

comparison of sequences by Blast analysis indicated that all 13 sequences were most similar (99.4 to 100%) to that of TPD phytoplasma, a subgroup 16SrIV-D strain (GenBank accession no. AF434989). By comparison, sequences from *S. mexicana* palm SmCx (Chicxulub), *C. nucifera* (Ticul), and *T. radiata* palm TrC2 (CICY) were most similar (99.9, 99.9, and 99.8%, respectively) to that of coconut LY phytoplasma (Juno-C2, accession no. AF498309), a known subgroup 16SrIV-A strain from Florida.

Phylogenetic analyses of 16S rDNA from representative phytoplasmas detected in *S. mexicana* and other palm species during this study all clustered together with other members of the coconut lethal yellows phytoplasma subclade (6) (Fig. 3). Tree branching patterns indicated that phytoplasma strains infecting *S. mexicana* from Ticul (GU473588), *P. sargentii* (GU473591), and *T. radiata* from CICY (*Tr1* GU473586) clustered together with other subgroup 16SrIV-D phytoplasma strains that included TPD and CPY whereas phytoplasma strains infecting the *S. mexicana* from Periferico (GU473585) and CICY (GU473587) were identical, sharing a single sub-branch, suggesting that they might represent a new subgroup within the coconut lethal yellows group. Sequences of phytoplasma strains infecting *C. nucifera* at Ticul (GU473590) and Chicxulub and *T. radiata* (*Tr*2 GU473592) from CICY, as well as *S. mexicana* at Chicxulub (GU473589), clustered together with the coconut LY phytoplasma strains from Florida and other members of subgroup 16SrIV-A (Fig. 3).

Symptom progression on *S. mexicana*. The most pronounced symptom overall in the foliar decay on mature, bearing *S. mexicana* palm trees was discoloration, necrosis, and loss of older leaves (Fig. 4A and B) which, at the outset, was essentially indistinguishable from that of natural leaf senescence associated with this palm species. However, as the disease developed, there was an increasing proportion of decaying and dying leaves compared with noninfected trees, progressively affecting younger leaves in the mid-crown and upper crown (Fig. 4C and D), finally leaving the palm without leaves. Although leaf decay was occurring, inflorescence and fruit production was also affected. Inflorescences that were already developed produced a decreased number of fruit (Fig. 4E) whereas those inflorescences that were developing showed atrophy, with smaller size and no fruiting at all (Fig. 4F). This symptom progression in adult bearing trees took more than 2 years, ending with the death of the palm.

Excessive foliar discoloration similar in appearance of that associated with mature diseased *S. mexicana* was also found on small, immature palm trees at Periferico in Merida, a site where mature *S. mexicana* trees were absent (Fig. 4H and I). All six palm trees with symptoms sampled at this site were judged to contain group 16SrIV phytoplasmas. However, phytoplasmas were not detected in any of the 11 other palm trees without discernible symptoms that were sampled at this site. Furthermore, during 3 years of periodic monitoring of the six infected trees, only one palm was observed to die, although leaf discoloration persisted on the remaining palm trees (Table 1).

When leaf, inflorescence, and fruit production were analyzed quantitatively on a monthly basis during an 18-month period, significant differences between known infected and uninfected mature palm trees were evident (Fig. 5). The average monthly number of green leaves supported by uninfected palm trees was 14.3 ± 0.68 whereas phytoplasma-infected palm trees retained 9.8 ± 0.80 green leaves, a significantly lower number (P = 0.05). Similarly, the number of fruit per inflorescence on uninfected trees was 4,850 ± 1,184, whereas the lower value of 2,212 ± 801 for infected trees was significantly different (P = 0.05). The measured decrease in the numbers of fruit per inflorescence appears to be a developmental response to infection by subgroup 16SrIV-D phytoplasmas rather than a result of natural fruit abortion which was not observed to occur in uninfected *S. mexicana* trees. In a related observation, the number of inflorescences that developed on each uninfected palm was 6.5 ± 0.58 and was significantly different (P = 0.05) from the value of 3.5 ± 0.52 obtained for infected palm trees. Furthermore, it is worth noting that none of the infected trees exhibiting symptoms from which this data were collected had died during the 18-month study period which ended in 2009, although foliar discoloration on these trees had continued to slowly intensify.

P. sargentii, T. radiata, or *C. nucifera* co-existing with *S. mexicana* in the Botanical Garden at CICY and at one or more of the other sampling sites showed evidence of pronounced leaf yellowing (Table 1). In the case of *P. sargentii* (Fig. 4J), symptom development was observed on only one palm and included fruit drop, excessive yellowing of leaves (Fig. 4K), and necrosis of inflores-

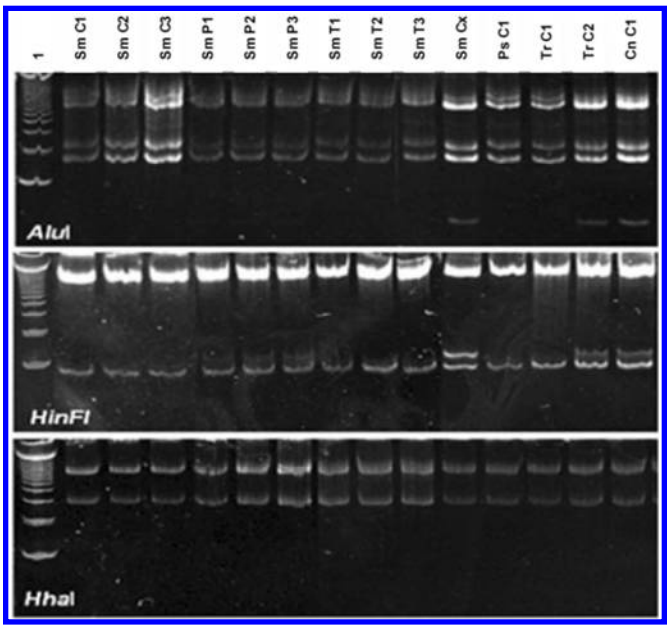

Fig. 2. Representative restriction fragment profiles of phytoplasmas 16S rDNA amplified from symptomatic palm trees by a nested polymerase chain reaction (PCR) assay incorporating rRNA gene operon primer pairs P1/P7 followed by 16SrIV group-specific primer pair LY16Sf/LY16Sr. PCR products were derived from *Sabal mexicana* (Sm), *Pseudophoenix sargentii* (Ps), *Thrinax radiata* (Tr), and *Cocos nucifera* (Cn) and digested with *Alu*I, *Hin*fI, or *Hha*I endonuclease. 1 = 100-bp DNA ladder.

Table 1. Analysis of four palm species with and without foliar discoloration symptoms at four sites in Yucatan State, Mexico for evidence of phytoplasma infection by a nested polymerase chain reaction assay employing phytoplasma universal rRNA operon primer pair P1/P7 followed by 16SrIV group-specific primer pair LY16Sf/LY16Sr

	Palm trees with positive detection (+), dead, and total analyzed (with symptoms)[a]											
	Sabal mexicana			***Pseudophoenix sargentii***			***Cocos nucifera***			***Thrinax radiata***		
Location	**+**	**Dead**	**Total**	**+**	**Dead**	**Total**	**+**	**Dead**	**Total**	**+**	**Dead**	**Total**
Mérida, CICY	8	4	21 (8)	1	1	6 (1)	0	0	7 (0)	2	1	9 (2)
Mérida, Periférico	6	0	17 (6)	…	…	…	…	…	…	…	…	…
Ticul	3	1	14 (6)	…	…	…	1	3	3 (3)	…	…	…
Chicxulub	1	0	1 (1)	…	…	…	1	1	1 (1)	3	1	7 (7)
Total	18	5	53 (21)	1	1	6 (1)	2	4	11 (4)	5	2	16 (9)

[a] Number of palm trees with symptoms is indicated in parentheses. The difference in the totals represents those palm trees without symptoms.

cence and spathe tissues (Fig. 4L), followed by mortality. Presence of a 16SrIV group phytoplasma in this palm was confirmed by nested PCR assay. Of the nine *T. radiata* palm trees in the Botanical Garden at CICY, phytoplasma infections were confirmed in only two symptomatic palm trees; phytoplasmas were not detected in any of seven symptomless palm trees. Similarly, of seven *T. radiata* trees with leaf yellowing sampled at Chicxulub, three subsequently tested phytoplasma positive. Among infected trees, mortality of one palm only occurred at each site. Although symptoms observed on three *C. nucifera* palm trees at Ticul and a single palm located at Chicxulub were typical of those described for LY disease by Zizumbo et al. (29), phytoplasmas were detected in only one palm at each location. All four trees sampled at these two locations died during the course of this study (Table 1).

Discussion

The occurrence of phytoplasmas of the 16SrIV group have been reported in several palm species (19); however, in some species, such as *S. mexicana* and *P. sargentii*, infection by these phytoplasmas or any other type have not been reported previously in the scientific literature. The main focus of this study was *S. mexicana* and the phytoplasmas invading it; however, other palm species (*P. sargentii, T. radiata*, and *C. nucifera*) that were coexisting with *S. mexicana* palm trees and showed leaf decay syndromes were also studied, with the intention of learning whether phytoplasmas were present and, if so, were the same or different strains. Palm trees studied included asymptomatic individuals because a previous report showed the occurrence of 16SrIV group phytoplasmas in asymptomatic *T. radiata* and *C. readii* palm trees (20). Here, we

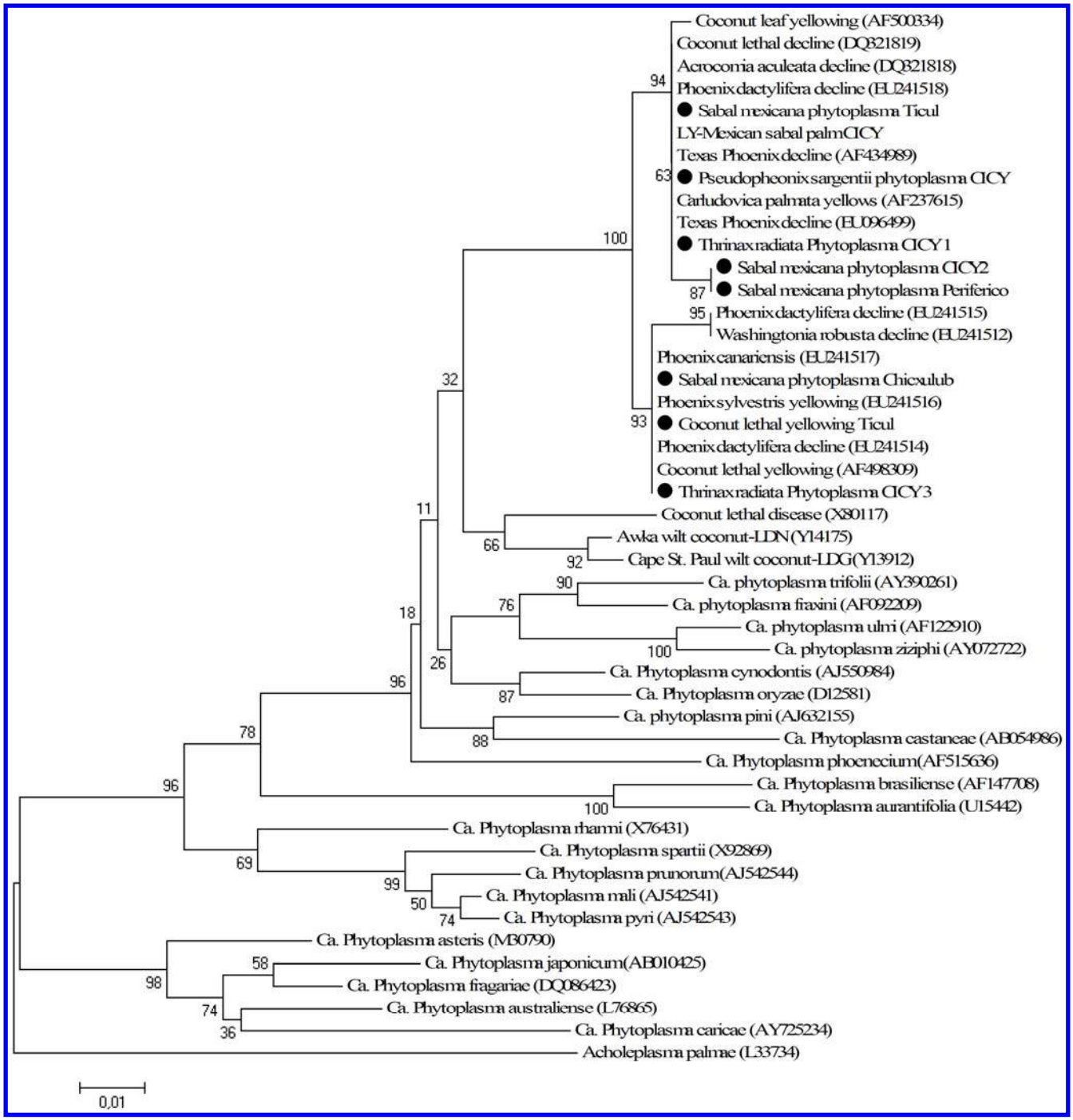

Fig. 3. Phylogenetic tree of 16S rRNA gene sequences from representative phytoplasmas in the coconut lethal yellows (16SrIV) group and other phytoplasma groups constructed by the neighbor-joining method. Phytoplasma strains sequences in this study are indicated by a black circle (●). Bootstrap values (used as an indicator of the reliability of the analysis) are shown on branches.

Fig. 4. Appearance and progression of foliar decay symptoms on *Sabal mexicana* infected with group 16SrIV phytoplasmas. **A,** Palm tree lacking discernible symptoms showing mostly green leaves; **B,** foliar discoloration starting on basal, older leaves; **C,** progressive discoloration of younger leaves in the mid- and upper crown; **D,** loss of most leaves from the crown prior preceding palm mortality; and **E,** reduction in fruit development on **F,** an inflorescence showing atrophy. **G** and **H,** Foliar decay symptoms in young *S. mexicana* palm trees infected with lethal yellowing phytoplasmas; **I,** detail. **J–L,** Foliar symptoms associated with phytoplasma infection of *Pseudophoenix sargentii*. **J,** Uninfected palm; **K,** infected palm; and **L,** yellow coloration of inflorescence sheath from an uninfected palm contrasting with the discolored sheath and emerging inflorescence from an infected palm.

report for the first time that *S. mexicana* and *P. sargentii* palm trees affected by leaf decay syndromes were associated with 16SrIV group phytoplasmas, as evidenced by nested PCR, RFLP assays, and nucleotide sequence analysis.

The symptoms observed in *S. mexicana* were similar to those caused by TPD (GenBank accession no. AF434989) in *Phoenix canariensis, P. dactylifera, P. sylvestris*, and *Syagrus romanzoffiana* (8,10). In *C. nucifera*, symptoms were similar to those already described in the literature (29). In the case of *Pseudophoenix sargentii* (spotted in only one single palm) and *T. radiata*, symptoms observed were the typical symptoms of LY disease, as were those reported for *C. nucifera*. Therefore, two patterns of symptoms were observed, one for *Sabal mexicana* and the other for *P. sargentii, T. radiata*, and *C. nucifera*, which seem to be independent of the type of phytoplasma infecting.

The identity of the phytoplasmas isolated from *S. mexicana* was studied. RFLP profiles obtained with restriction enzymes *Alu*I and *Hin*fI showed that it could be hosting two phytoplasma strains of the 16SrIV group. In one case, profiles were the same observed for these enzymes for LY-Fla phytoplasmas and 16SrIV-A subgroup (7,20); and, in the second case, for TPD and CPY phytoplasmas and the 16SrIV-D subgroup (10). The first one was found in only 1 of the 10 positive *S. mexicana* palm trees analyzed (GU473589). In the other nine trees, profiles were of subgroup D and coexistence of both strains was not found in any of the palm trees analyzed. These identities were supported by analyses of the amplicons obtained by nested PCR by nucleotide-nucleotide BLAST alignments using the NCBI database. However, phylogenetic analysis showed one more strain group for phytoplasmas from an *S. mexicana* palm at CICY (GU473585) and one at Periferico (GU473587), both clustered together in one separate branch, and then probably representing a new 16SrIV subgroup. The results also suggest that there is a more frequent association of subgroup D phytoplasmas than subgroup A phytoplasmas with *S. mexicana* palm trees, and that the 16SrIV group phytoplasmas can invade young and adult *S. mexicana* trees.

When analysis of samples from other palm species with leaf decay symptoms that were co-existing with *S. mexicana* palm trees was carried out, 16SrIV group phytoplasmas were also detected in them. In CICY's Botanical Garden, we found a *T. radiata* palm with 16SrIV-A phytoplasmas but also another *T. radiata* palm and a *P. sargentii* palm with 16SrIV-D phytoplasmas the same as those found in *S. mexicana* trees. Occurrence of 16SrIV-A phytoplasmas in *T. radiata* has already been reported, although in asymptomatic palm trees (20), but the 16SrIV-D phytoplasmas have not been reported before in this species; neither these phytoplasmas nor any other in *P. sargentii* palm trees. Therefore, these results show that 16SrIV-D phytoplasmas can be hosted by different palm species in Yucatan State. How extensive this could be in the species studied and whether it occurs in others remains to be determined. Interestingly, a *C. nucifera* palm and an *S. mexicana* palm in Ticul located at about 10 m from each other were infected by 16SrIV group phytoplasmas but, in the first palm, it was subgroup A and, in the second palm, subgroup D, an observation that is consistent with the fact that, in Yucatan State, *C. nucifera* palm trees have been associated only with subgroup A phytoplasmas and never with subgroup D phytoplasmas. However, in the case of the Mexican Pacific Coast, *C. nucifera* trees have been associated with subgroup D phytoplasmas (7). Therefore, it will be important to carry out a more extensive survey of phytoplasmas occurring in *C. nucifera* trees in Yucatan State to determine whether subgroup D phytoplasmas can also be found in this species and, if that occurs, what the significance could be.

Regarding the source of the *S. mexicana* 16SrIV-D phytoplasmas, we may speculate about two possible origins. One could be related to the TPD phytoplasmas found in Texas and Florida (8,10) because there is a very high homology between *S. mexicana* 16SrIV-D phytoplasmas and TPD phytoplasmas. We can also consider the CPY phytoplasmas isolated from *C. palmata* plants (3), because there is also a very high homology between *S. mexicana* 16SrIV-D phytoplasmas and CPY phytoplasmas. The original CPY phytoplasma outbreak was reported in Calkini, Campeche (3), a location close to Ticul, one of the sites where *S. mexicana* trees were found to be infected by 16SrIV-D phytoplasmas. Therefore, the last possibility could be more likely because of the geographic proximity. Also, it is important to consider that the development of symptoms in *S. mexicana* infected by 16SrIV-D phytoplasmas is lengthy and the mortality is low according to casual observations during sampling in different sites, suggesting that the subgroup 16SrIV-D phytoplasma is a native strain rather than an exotic strain.

In the case of the 16SrIV-A phytoplasma found in a single *S. mexicana* palm in Chicxulub, it might have been transmitted from other palm species in this location that were affected by this phytoplasma, such as *T. radiata* and *C. nucifera.* How 16SrIV-A and 16SrIV-D phytoplasmas could be vectored into *S. mexicana* palm trees was not studied but *Myndus crudus*, the cixiid vector of LY (12), was found visiting *S. mexicana* trees in all the locations studied; therefore, it would be important to consider this insect for future evaluation as vector of the phytoplasma strains found in *S. mexicana* and other palm species reported here. This also might explain the geographic spread of the association of 16SrIV group phytoplasmas with *S. mexicana*. This association was found in CICY's premises in Merida; in Periferico, also in Merida about 10

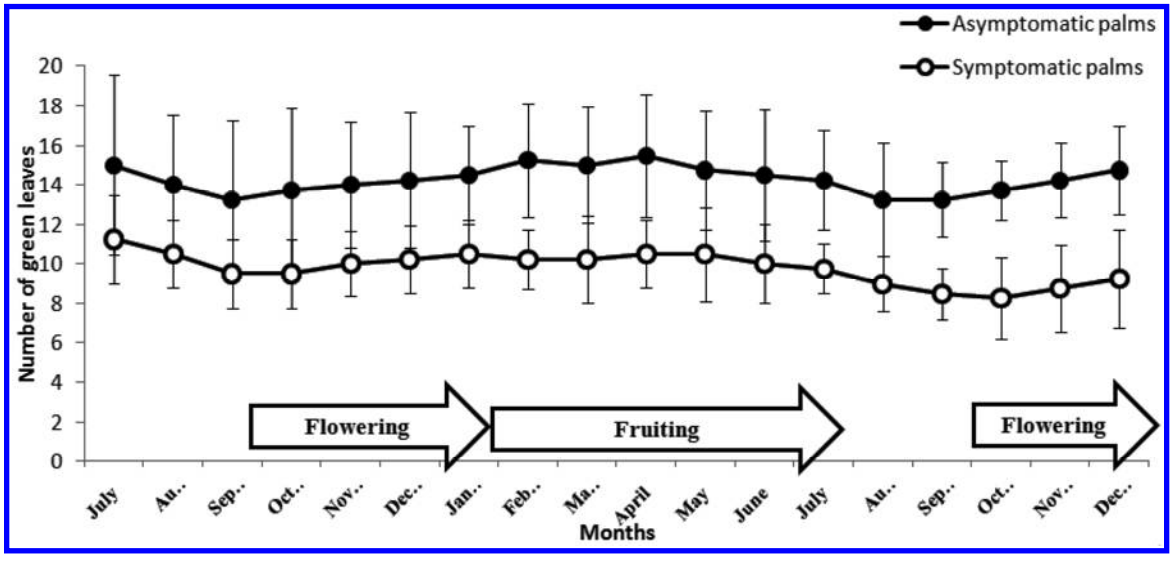

Fig. 5. Average number of green leaves on phytoplasma-infected and uninfected *Sabal mexicana* over an 18-month period (n = 4).

km away; and in Chicxulub and Ticul, located 40 km north and 100 km south respectively, of Merida. No further locations were explored but, considering what was found, it is reasonable to assume that a wider distribution could be possible because *S. mexicana*, together with *P. sargentii*, *Cocothrinax readii*, and *T. radiata*, are palm species widely distributed along the coasts of the Yucatan Peninsula (23). Then, as previously proposed for *C. readii* and *T. radiata* (20,21), *S. mexicana* trees might be considered to be a potential permanent source of 16SrIV phytoplasmas inoculum and, thus, a threat to *Cocos nucifera* and other palm species in Yucatan State. This is based on the following: (i) *S. mexicana* trees are widely distributed along the Yucatan Peninsula coasts; (ii) the 16SrIV-A and 16SrIV-D subgroup phytoplasmas (and probably another one) have been found in *S. mexicana* trees; (iii) this association is widely distributed geographically; and (d) although some palm trees were positive (18 of 21 symptomatic) and some died (n = 5), most *S. mexicana* trees in locations were not affected. Also, the finding of 16SrIV-D subgroup phytoplasmas in *T. radiata* and *P. sargentii* palm trees opens the possibility that these palm species could also be sources of inoculum of these phytoplasmas in Yucatan. Therefore, it will be necessary to continue researching these associations of 16SrIV phytoplasmas with *S. mexicana* and the other palm species to fully understand their importance as permanent sources of inoculum of phytoplasmas, and how extensive a threat they might pose to palm trees of social and economic importance such as *C. nucifera*.

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