Palm Phytoplasmas in the Caribbean Basin

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Lethal yellowing (LY) of *Cocos nucifera* and numerous other palm species is the dominant phytoplasma disease occurring in the Caribbean Basin. However, the phytoplasma strain that causes LY is not the only one affecting palms in this region. This review briefly discusses what are phytoplasmas, how they are identified and classified, and which palm phytoplasma strains are present in the Caribbean Basin and where they have been detected.

Palm diseases caused by phytoplasmas can now be found throughout the humid tropics, but this review is limited to the phytoplasma diseases occurring on palms in the Caribbean Basin. However, defining the Caribbean Basin is difficult, and there appears to be no definitive definition. Therefore, for the purposes of this review, we use the following (Wikipedia 2012):

> "The Caribbean Basin is generally defined as the area running from Florida westward along the Gulf coast, then south along the Mexican coast through Central America and then eastward across the northern coast of South America. This region includes the islands of the archipelago of the West Indies. Bermuda is also included within the region even though it is in the west-central Atlantic, due to its common cultural history created by European colonization of the region, and in most of the region by the presence of a significant group of African descent."

Phytoplasmas

Phytoplasmas are unculturable, cell wall-less bacteria that belong to the class Mollicutes (Kirkpatrick 1992, Lee et al. 2000, Bai et al. 2006, Bertaccini 2007). These very small bacteria alternate passage between plant and insect hosts, in which they propagate and persist. Phytoplasmas inhabit phloem sieve tubes of their plant hosts and depend on transmission from plant to plant by phloem-feeding insect vectors of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids (Weintraub & Beanland 2006). This pathogen group is associated with over 1000 plant diseases, causing a wide variety of symptoms and potentially plant death. However, some plant species are tolerant of phytoplasmas and therefore show mild or no

symptoms. Because phytoplasmas are nutritionally fastidious, and thus far unculturable, their taxonomic characterization is limited mainly to molecular-based methods.

Before a comprehensive classification for phytoplasmas was devised, phytoplasmas were often named according to primary plant host and main symptom they caused. The shortfall of this system was that molecularly distinct phytoplasmas that cause the same symptoms were usually assigned the same name. Murray and Schleifer (1994) proposed the '*Candidatus*' system for assigning binomial names to incompletely described prokaryotes. This system was adopted for genus and species descriptions of phytoplasmas for taxonomic purposes (IRPCM 2004). An alternative scheme for identification and classification of phytoplasmas based on restriction fragment length polymorphism (RFLP) analysis of 16Sr RNA gene and ribosomal protein gene sequences has been widely used also (Lee et al. 1998, Seemüller et al. 1998, Martini et al. 2007, Wei et al. 2007). According to the most recent classification scheme (Wei et al. 2007), phytoplasmas may be differentiated into 28 major groups, known as 16Sr groups, with numerous sub-groups or strains within some of the major groups. Using the species concept, two phytoplasma strains are the same species if they share at least 97.5% of their 16S rRNA gene (IRPCM 2004, Harrison et al. 2011). However, if two such strains (that share more than 97.5% of their 16S rRNA) are vectored by different insects, or have different hosts or behave differently in the same host, or are molecularly distinct based on DNA hybridization tests or can be differentiated by serotyping or polymerase chain reaction (PCR) assays, then these two strains warrant separate '*Ca*. Phytoplasma species' designations.

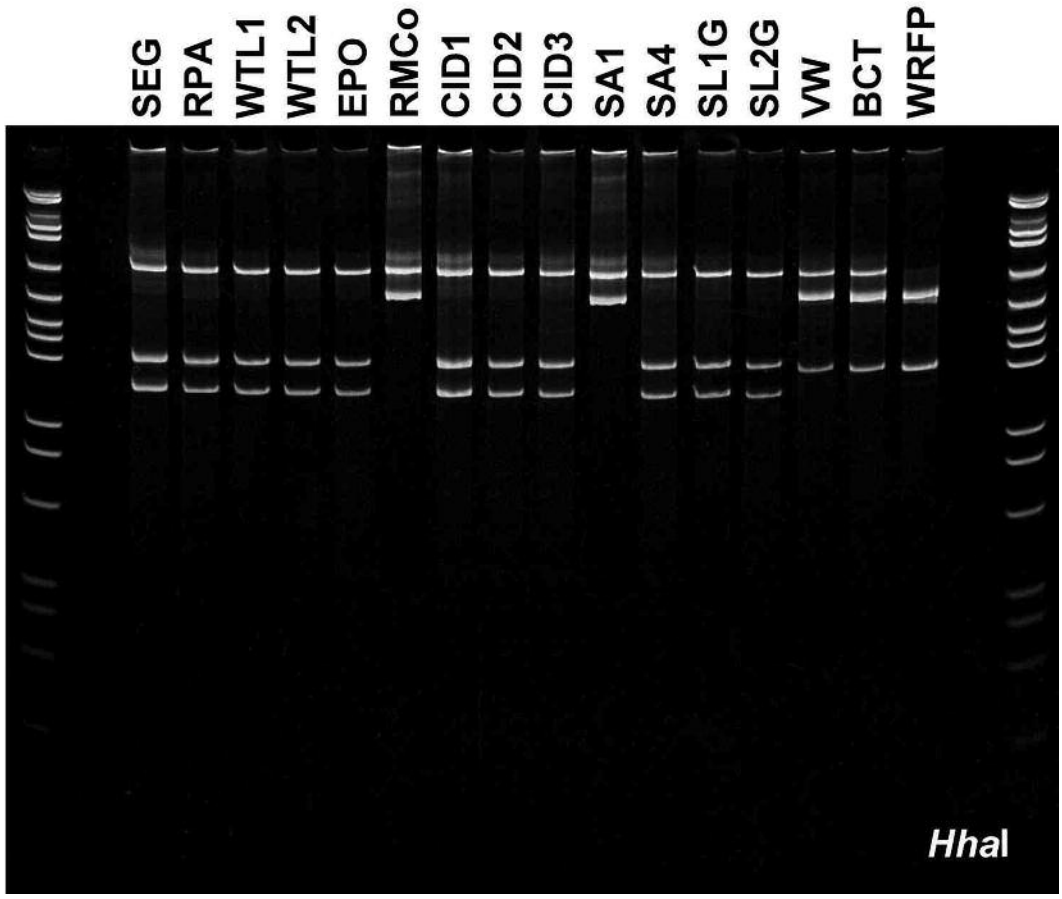
Sampling and Detection of Palm Phytoplasmas

Because phytoplasmas are unculturable, molecular methods are currently used to detect and identify them in tissues of affected palms. While immature leaf bases are usually the most reliable tissue for detection of phytoplasmas, the most common non-destructive method for obtaining samples from palms for phytoplasma detection uses trunk tissue (Oropeza et al. 2011, Harrison et al. 2013). With this method, a wood drill bit is heated with a blow torch to eliminate any contaminating DNA, cooled with water and then used to drill into the palm trunk. As the drill bit is moved in and out of the trunk, internal trunk tissue shavings are collected in a self-sealing plastic bag; approximately 3 grams of material is required. For palms that retain their old leaf bases, it is important that the drill bit is sufficiently long to obtain trunk tissue and not old leaf base tissue.

DNA is extracted from this trunk tissue and then used for nested PCR assays (Harrison & Oropeza 2008, Harrison et al. 2013). In general, the primers used for the first PCR are phytoplasma universal primer pair P1/P7 (Smart et al. 1996)), and primer pair LY16Sf/LY16Sr is used for the second PCR (Harrison et al. 2002). These primer pairs amplify group 16SrIV phytoplasma strains in a relatively group-specific manner (Harrison & Oropeza 2008). Detection specificity is enhanced by using primer pair P1m/LY16-23Sr followed by primer pair LY16Sf2/LY16-23Sr2 (Harrison et al. 2008). The resulting PCR products are viewed by agarose gel electrophoresis. Positive results, which indicate that a group 16SrIV phytoplasma has been detected, are then subjected to enzymatic digestion using specific restriction enzymes that "cut" the DNA. Products of the restriction digests are separated by nondenaturing polyacrylamide gel electrophoresis (Fig. 1) (Harrison et al. 2013). While the PCR products are sequenced for supporting information, the fragment patterns resulting from RFLP analysis provide unequivocal visual evidence about phytoplasma identity.

Group 16SrIV phytoplasmas

Group 16SrIV phytoplasmas cause lethal yellowing (LY), LY-like and lethal decline symptoms on palms. This group of phytoplasmas is commonly referred to as the Coconut Lethal Yellows Group. Based on symptom description, some of the diseases they cause, such as LY, have been known for more than one-hundred years in the Caribbean Basin. While symptoms vary among palm species and even among cultivars within a species, there are common elements that aide in field diagnosis of these diseases. Initial symptoms include flower necrosis and premature fruit fall. In *Cocos nucifera* (coconut palm), these symptoms can be detected all year because *C. nucifera* flowers and fruits all year. With other palm genera, such as *Phoenix*, these initial symptoms are unreliable because the palm only flowers and fruits once each year. Another early symptom is premature chlorosis and necrosis of the oldest leaves. For most



1. Visualization of the fragment profiles following endonuclease digestion of phytoplasma rDNA products amplified from symptomatic palms infected with a group 16SrIV phytoplasma. RMCo and SA1 represent the pattern obtained for strain A. SEG, RPA, WTL1, WTL2, EPO, CID1, CID2, CID3, SA4, SL1G and SL2G represent the pattern obtained for strain D. WRFP represents the pattern obtained for strain F. VW and BCT represent the pattern obtained for a co-infect of strains A and F. SA1 and SA4 are samples from two different *Phoenix canariensis* located in Manatee County, Florida, USA.

palm species, there is progressive discoloration of the leaves from the bottom to the top of the canopy, with the spear leaf being the last leaf to die. With *Phoenix, Sabal* and a few other genera, the spear leaf dies prematurely, prior to the youngest leaves dying.

Early reports in the Caribbean Basin of mortality of palms due to yellowing symptoms were made in the Cayman Islands (1834), Cuba (1870s) and Jamaica (1870s) (Fawcet 1891, De La Torre 1906, Johnston, 1912, Martyn 1945, Nutman & Roberts 1955, Ollagnier & Weststeijn 1961, Maramorosch 1978, McCoy et al. 1983). After these first records, palm lethal yellows were also reported in the Dominican Republic (Ciferri & Cicarone 1949, Schieber & Hichez-Frias 1970), Haiti (Leach 1946), Bahamas (Leach 1946) and Florida in the USA (Martinez & Roberts 1967, Thomas 1979). Other outbreaks were also reported in the Yucatán Peninsula of Mexico (McCoy et al. 1983, Cardeña et al. 1991), Belize (Escamilla et al. 1994, Harrison & Oropeza 1997), Guatemala (Mejía et al. 2004) and Honduras (Ashburner et al. 1996). The most recent observations of palm lethal yellows have been in Antigua (Gordon 2012) and St. Kitts & Nevis (Myrie et al. 2006, Myrie et al. 2012).

Most lethal yellowing disease reports cited above were made on *Cocos nucifera*. In Florida and Jamaica, LY epidemics killed most of the 'Jamaican Tall' variety of *C. nucifera* from the 1970s until the 1990s. However, in Florida, where palms are a dominant element in the landscape, numerous palm species have been documented to be affected by LY (Harrison & Jones 2004). During the late 1970s, McCoy et al. (1980) reported on a disease epidemic

2. Current Caribbean Basin locations of five strains in the 16SrIV phytoplasma group.

caused by a LY-type phytoplasma occurring on *Phoenix canariensis* (Canary Island date palm) and *P. dactylifera* (date palm) in the Brownsville and Rio Grande Valley in southern Texas. In 2002, Harrison et al. reported detecting a 16SrIV phytoplasma in *P. canariensis* in Corpus Christi, Texas. Symptoms on *P. canariensis* resembled those reported previously by McCoy et al. (1980). Later records of LY-type diseases on palms were made on *P. canariensis, P. dactylifera, P. sylvestris* (wild date palm), *Syagrus romanzoffiana* (queen palm) and *Washingtonia robusta* (Mexican fan palm) in west-central Florida (Harrison et al. 2008). A lethal decline of *Sabal palmetto* was also recorded in west-central Florida in 2008 (Harrison et al. 2009). This latter report was the first time that a phytoplasma disease was documented for a palm native to the Caribbean Basin, although a recent study completed in the Yucatán peninsula of Mexico has expanded this list (Vázquez-Euán et al. 2011).

Occurrence of 16SrIV sub-group phytoplasmas in the Caribbean Basin

Subgroup 16SrIV-A

This subgroup is the strain that causes the palm disease commonly referred to as lethal yellowing (LY). Early reports of this disease were made prior to the use of molecular classification schemes, but subsequent studies have confirmed the pathogen in most of these countries. Lethal yellowing has been observed on *Cocos nucifera* in Antigua, Bahamas, Belize, Cayman Islands, Cuba, Dominican Republic, Guatemala, Haiti, Honduras, Jamaica, Yucatán Peninsula of Mexico, St. Kitts & Nevis and Florida (USA). In Florida, subgroup 16SrIV-A strains have been detected as far north as Polk county in 40 palm species (see Harrison & Jones 2004 for partial list) but never in a palm species native to the Caribbean Basin. However, recent studies in the Yucatán peninsula of Mexico detected this subgroup in *Cocothrinax readii* (thatch palm), *Sabal mexicana* and *Thrinax radiata* (thatch palm), three species native to the Caribbean Basin (Narváez et al. 2006; Vázquez-Euán et al. 2011).

Subgroup 16SrIV-B

This strain has been detected in *Acrocomia aculeata* (grugru palm) and *Cocos nucifera* in Honduras (ca. 150 km from the Atlantic coast) (Roca et al. 2006) and *C. nucifera* in Mexico's Yucatán Peninsula (Harrison & Oropeza 1997).

Subgroup 16SrIV-C

While this phytoplasma subgroup has not been detected in the Caribbean Basin, it is noted here, lest readers feel we do not know the alphabet. It is known to cause a lethal disease of *Cocos nucifera* in East Africa (Hodgetts et al. 2008). However, this strain illustrates the problem with using the classification scheme of Wei et al. (2007), which relies on RFLP analysis of a 1.25 kb fragment of the 16Sr gene, a relatively short fragment. When analysis of a larger portion of the 16Sr gene sequence is conducted, this strain represents a phytoplasma that is phylogenetically distinct from other group 16SrIV phytoplasmas group and from strains affecting *C. nucifera* in western Africa (Tymon et al. 1998).

Subgroup 16SrIV-D

Confirmation of this strain did not occur until early 2000s, when Harrison et al. (2002) determined that the phytoplasma disease affecting *Phoenix canariensis* in Corpus Christi, Texas was caused by a subgroup 16SrIV-D strain. Symptoms observed at that time resembled those on *P. canariensis* and *P. dactylifera* during an epidemic in the Brownsville and Rio Grande Valley in southern Texas during the late 1970s (McCoy et al., 1980). Although no phytoplasma DNA existed from that time period, subsequent surveys in Texas have detected this strain in Bexar, Cameron, Hidalgo, Kleberg, Nueces and Willacy counties in Texas occurring in *Phoenix canariensis, P. dactylifera* and *Sabal palmetto* (Ong & McBride, 2009).

As noted previously, this strain was subsequently detected in west-central coastal Florida when *Phoenix* spp. and then *Sabal palmetto* began dying with LY-like symptoms (Harrison et al. 2008, 2009). To date, subgroup 16SrIV-D strains have been identified in Florida in eight palm species: *Phoenix canariensis, P. dactylifera, P. reclinata, P. roebelenii* (pygmy date palm), *P. sylvestris, Sabal palmetto, Syagrus romanzoffiana,* and ×*Butiagrus nabonnandii* (*Butia odorata* × *S. romanzoffiana*). To date, *Phoenix* spp. have been most widely affected in Florida with confirmed cases of diseases in Charlotte, Duval, Highlands, Hillsborough, Indian River, Lake, Lee, Manatee, Orange, Palm Beach, Pinellas, Polk and Sarasota counties. Both subgroup16SrIV-A and 16SrIV-D strains induce the same symptoms on *Phoenix* palms. Therefore, subgroup 16SrIV-D strains may occur in other Florida counties too, but until its presence has been confirmed in affected palms, it is not counted as an actual occurrence. For *S. palmetto*, detection of infected palms has been limited to Charlotte, DeSoto, Hardee, Hillsborough, Manatee, Polk and Sarasota counties in Florida.

In a recent study conducted in the Yucatán peninsula of Mexico, subgroup 16SrIV-D phytoplasmas were detected in *Pseudophoenix sargentii* (buccaneer palm), and in both *Sabal mexicana,* and *Thrinax radiata*, palms native to this area (Váquez-Euán et al. 2011). What was most notable about the observations of *S. mexicana* is that only 5 of 18 phytoplasma-positive palms died during the course of the 18-month study period. However, it was also noted that the number of green leaves on affected palms varied due to phytoplasma infection, with *S. mexicana* supporting 14.3 leaves on healthy palms but only 9.8 leaves on infected palms. Subgroup 16SrIV-D phytoplasmas were also recently detected in Puerto Rico in *Carpentaria acuminata, Caryota mitis* (clustered fishtail palm) and a *Roystonea* sp. (royal palm) (Rodrigues et al. 2010; sequences deposited in GenBank by N. Harrison). More information is needed from Puerto Rico regarding this strain, hosts and symptom expression.

Subgroup 16SrIV-E

This subgroup strain has only been detected in localized outbreaks of disease among *Cocos nucifera* on the southern coast of the Dominican Republic (Martinez et al. 2007). The phytoplasma is more closely related to strain 16SrIV-B than it is to strain 16SrIV-A.

Subgroup 16SrIV-F

In the process of surveying for 16SrIV-D phytoplasma in west-central Florida, Harrison et al. (2008) detected another, previously unknown 16SrIV subgroup phytoplasma, subsequently labeled strain F. It was detected in two *Washingtonia robusta* and in two *Phoenix dactylifera*. So far, subgroup 16SrIV-F strains have not been detected in any other palm samples even though numerous other palm samples have been received from the general vicinity of these palms. Interestingly, the two *P. dactylifera* were also co-infected with subgroup 16SrIV-A phytoplasmas, which highlights the importance of using RFLP analysis in addition to DNA sequencing to separate and identify phytoplasmas.

Summary

While the focus of palm phytoplasma diseases in the Caribbean Basin has always been on lethal yellowing of *Cocos nucifera* caused by subgroup 16SrIV-A strains, we now know that: 1) at least four more 16Sr IV subgroup strains occur in this region (Fig. 2); 2) the same palm species can be infected by multiple strains (e.g., *Phoenix canariensis* is susceptible to both strain A and strain D); 3) mixed infections of strains in the same palm are possible; and 4) infection by these phytoplasmas may not always cause the palm to die. The latter observation is especially intriguing, as it may mean many more palms, especially palms native to the Caribbean Basin, are hosts to phytoplasmas but either remain asymptomatic or the mild symptoms are largely overlooked because they were presumed to be due to another biotic or abiotic problem. Much is yet to be learned about these diseases in palms and in their insect vectors.

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