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REVIEW ARTICLE

# Phytoplasmas associated with date palm in the continental USA: three 16SrIV subgroups

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## ABSTRACT

Only one major group of phytoplasmas, namely group 16SrIV, has been identified in date palms in the continental United States, and only in the states of Florida and Texas where date palms are used for aesthetic purposes in the landscape and not for date production. While strains belonging to three 16SrIV group have been detected in Florida date palms (16SrIV-A, 16SrIV-D and 16SrIV-F), only subgroup 16SrIV-D strains have been detected in Texas date palms. The subgroup 16SrIV-D phytoplasmas identified in Florida and Texas appear to be genetically the same. Field symptoms caused by this group of phytoplasmas in date palms is described, along with the molecular techniques used to detect the phytoplasma in palm tissue and to identify the subgroup detected. Preventive management in the landscape is based on use of resistant palm material and liquid trunk injection of the antibiotic oxytetracycline HCl into susceptible palms.

**Keywords:** *Phoenix dactylifera*; Date palm; Phytoplasma; Subgroup 16SrIV-; Subgroup 16SrIV-D

## INTRODUCTION

Phytoplasmas are unculturable, cell wall-less bacteria that depend on transmission from plant to plant by phloem-feeding insect vectors of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids (Bai et al., 2006; Bertaccini, 2007; Kirkpatrick, 1992; Lee et al., 2000; Weintraub and Beanland, 2006). Phytoplasmas belong to the class Mollicutes (Bai et al., 2006; Bertaccini, 200; Kirkpatrick, 1992; Lee et al., 2000), and using the most recent classification scheme, phytoplasmas are differentiated into major groups and sub-groups of strains based on RFLP analysis of the 16S rRNA gene (Lee et al., 1998; Martini et al., 2007; Wei et al., 2007). 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). Using the species concept, two phytoplasma strains are the same species if they share at least 97.5% of their 16S rRNA gene (Harrison et al., 2011; IRPCM, 2004). However, if two such strains (that share more than 97.5% of their 16S rRNA) are vectored by different insect species, or have different plant hosts or behave differently in the same host plant, 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.

Group 16SrIV phytoplasmas cause lethal yellowing (LY), LY-like and lethal decline symptoms on palms. This group of phytoplasmas is collectively referred to as the Coconut Lethal Yellows Group and member strains are primarily distributed in the Caribbean Basin (Ntushelo et al., 2013). Based on symptom description, some of the diseases they cause, such as LY, have been known for more than one-hundred years on coconut (*Cocos nucifera* L.) in this region. Lethal yellowing (LY) was first reported in the Florida Keys in the 1960s on coconut (Martinez and Roberts, 1967) and on mainland southern Florida during the 1970s (Thomas, 1979), after which numerous palm species have been documented to be affected by LY, including date palm (*Phoenix dactylifera* L.) (Harrison and Jones, 2004). With the advent of molecular techniques and the classification scheme identified above, the LY phytoplasma has been designated as subgroup 16SrIV-A. In a preliminary study, Howard (1992) evaluated the date palm cultivars for their relative susceptibility to the LY phytoplasma and

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determined cultivars Deglet Noor, Zahidi and Thoory to be more susceptible than cultivars Medjool and Halawy following 7 years of natural exposure to the disease.

During the late 1970s, McCoy et al. (1980) reported on a disease epidemic caused by an LY-type phytoplasma occurring on Canary Island date palm (*Phoenix canariensis* Chabaud) and date palm in the Rio Grande Valley of southwestern Texas. Since this disease epidemic occurred prior to the use of molecular techniques for phytoplasma identification, the phytoplasma was simply compared with the LY phytoplasma at that time based on microscopy and the symptoms that it caused.

In 2002, Harrison et al. reported detecting a 16SrIV phytoplasma in Canary Island date palm in Corpus Christi, Texas, a location on the southeastern coast of Texas that is approximately 260 km from the earlier disease epidemic of the late 1970s. The palm symptoms in Corpus Christi closely resembled those described previously by McCoy et al. (1980). This phytoplasma was found to be genetically distinct from the subgroup 16SrIV-A that causes LY and was subsequently classified as a member of a new subgroup, 16SrIV-D.

In 2005-2006, LY-type diseases were reported occurring on Canary Island date palm, edible date palm, wild date palm [*Phoenix sylvestris* (L.) Roxb.], queen palm [*Syagrus romanozoffiana* (Cham.) Glassman], and cabbage palm (*Sabal palmetto* [(Walter) Lodd. Ex Schult. & Schult. f.] in coastal, west central Florida (Harrison et al., 2008; Harrison et al., 2009). LY had never been previously reported from this region of Florida. The phytoplasma causing these diseases was determined to be a subgroup 16SrIV-D strain, the same subgroup causing LY-type diseases in Texas. However, in this same area of Florida, a third 16SrIV group phytoplasma, subgroup 16SrIV-F, was also detected in three declining Mexican fan palms (*Washington robusta* H.A. Wendl.), and in two date palms either singly, or as mixed infections with both 16SrIV-A and 16SrIV-F strains (Fig. 1). However, no further information has since been acquired about this new subgroup phytoplasma and so it will not be discussed further.

Coastal, west central Florida (e.g., Tampa, Florida) is approximately 1500 km east of Corpus Christi, Texas, with the Gulf of Mexico separating these two port cities. Few palms are transported from Texas to Florida, but other plant material could be transported. Recently, subgroup 16SrIV-D was detected in Canary Island date palms in New Orleans, Louisiana (Singh, 2014), which is another port city along the Gulf of Mexico.

Although the subgroup 16SrIV-D strain was detected by one of the authors (Harrison) from date palm samples

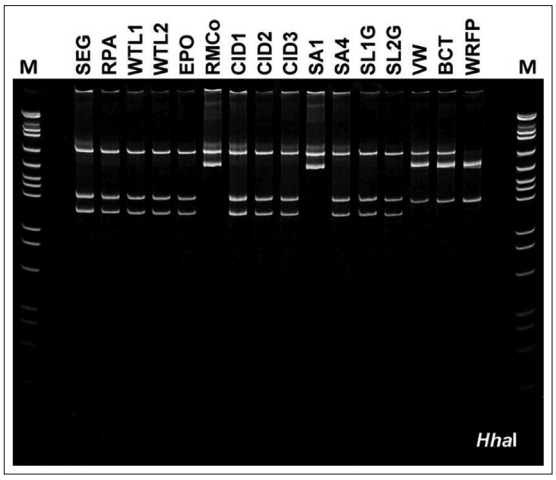


**Fig 1.** Comparison of representative fragment profiles following endonuclease digestion with *HhaI* of phytoplasma rDNA products (1.6 kb) amplified from symptomatic palms by nested PCR with rRNA gene operon primer pairs P1m/LY16–23Sr and then LY16Sf2/LY16–23Sr2. PCR products were derived from *Phoenix dactylifera* (RPA, VW and BCT) and *Washingtonia robusta* (WRFP), while all other products were from *Phoenix canariensis*. pGEM, molecular size (bp) markers in descending order: 2465, 1605, 1198, 676, 517, 460, 396, 350, 222, 179, 126, 75, 65, 51 and 36. Palms RMCo and SA1 were infected with 16SrIV-A phytoplasma. Palms SEG, RPA, WTL1, WTL2, EPO, CID1, CID2, CID3, SA4, SL1G, and SL2G were infected with16SrIV-D phytoplasma. Palm WRFP was infected with 16SrIV-F phytoplasma. Palms VW and BCT were co-infected with 16SrIV-A and 16SrIV-F.

received from a private grower in Riverside County, California in 2014, subsequent date palm samples obtained directly by the California Department of Food and Agriculture from the grower were judged negative for infection by this phytoplasma. At this time, the 16SrIV-D phytoplasma has not been officially detected in California (Chitambar, 2015).

To summarize, only one major group of phytoplasmas, namely group 16SrIV, has been identified in date palms (*Phoenix dactylifera*) in the continental United States, and only in the states of Florida and Texas where date palms are used for aesthetic purposes in the landscape and not for date production. While strains belonging to three 16SrIV group have been detected in Florida date palms (16SrIV-A, 16SrIV-D and 16SrIV-F), only subgroup 16SrIV-D strains have been detected in Texas and Louisiana date palms. The subgroup 16SrIV-D phytoplasmas identified in Florida and Texas appear to be genetically the same.

Based on our observations in Florida where date palms are abundantly used in the landscape throughout the state, date palms appear to be more susceptible to subgroup 16SrIV-D than to subgroup 16SrIV-A, but there is no quantitative data collected so far to support this opinion.

## OTHER PHYTOPLASMAS OF DATE PALM

While only group 16SrIV phytoplasmas are associated with diseases of date palms in the continental USA, other phytoplasmas have been detected in date palms elsewhere in the world. Phytoplasma diseases of date palms have been reported from Egypt, Kuwait, Saudi Arabia and Sudan (Al-Awadhi et al., 2002; Alhudaib et al., 2008; Alhudaib et al., 2014; Al Khazindar, 2014; Ammar et al., 2005; Cronjé et al., 2000; El-Zayat et al., 2002). Thus far, the phytoplasma groups implicated in these disease have been identified as group 16SrI (Egypt and Saudi Arabia) (Alhudaib et al., 2008; Al Khazindar, 2014), 16SrII (Saudi Arabia) (Alhudaib et al., 2014) and 16SrXIV (Sudan) (Cronjé et al., 2000).

## GEOGRAPHIC RANGE OF SUBGROUP 16SRIV-D

While the 16SrIV-A phytoplasma has been known to occur in Florida since the 1960s, it is not known when the 16SrIV-D phytoplasma arrived in Florida. While first detected in 2005-2006, considerable time would have been necessary for both the pathogen and the vector populations to establish and for the disease incidence to increase when it was first detected. The disease caused by subgroup16SrIV-D is commonly referred to in Florida as Texas Phoenix palm decline (TPPD). In Texas, it is referred to as date palm lethal decline.

As of August 2015, the presence of the 16SrIV-D phytoplasma has been confirmed in symptomatic palms in Florida in the following counties: Alachua, Broward, Charlotte, De Soto, Duval, Highlands, Hillsborough, Indian River, Lake, Lee, Manatee, Palm Beach, Pinellas, Polk, Orange, and Sarasota. Not all of the detections were in date palms.

In Texas, the 16SrIV-D phytoplasma has been confirmed in the following counties: Cameron, Harris, Hidalgo, Kleberg, Nueces and Willacy (Ong and McBride, 2009; Texas Department of Agriculture, nd). In Louisiana, the 16SrIV-D phytoplasma has been confirmed only in Orleans Parish (Singh, 2014). Again, not all of the detections were in date palms.

## PLANT AND INSECT HOSTS OF SUBGROUP 16SRIV-D

Currently, the primary susceptible palm hosts of the 16SrIV-D phytoplasma are *P. canariensis*, *P. dactylifera, P. sylvestris* and *S. palmetto*. Also susceptible, but with fewer reports are *P. reclinata* and *S. romanzoffiana*. This phytoplasma has also been detected in *P. roebelenii* and *Butiagrus nabonnandii*, but only one time for each species (Jeyaprakash et al. 2011; Harrison, personal observation).

The planthopper *Haplaxius* (formerly *Myndus*) *crudus* is the vector for subgroup 16SrIV-A in Florida (Howard et al., 1983). The identity of the vector species of subgroup 16SrIV-D is presently unknown, but *H. crudus* is suspected (Halbert et al., 2014). However, this planthopper was not detected in surveys completed in Texas in areas where Texas Phoenix palm decline had occurred (Meyerdirk and Hart, 1982). It should be noted that *H. crudus* completes its life cycle in grasses, including commonly used turfgrasses in the landscape (Howard, 1990). Thus, even if infected palm hosts are not moved, movement of turfgrass sod and possibly other ornamental grass material could also move infected vectors to a new site.

How far and how quickly TPPD will spread to other Florida counties is unknown. However, since movement of palms and other plant material occurs widely in Florida, it is likely people will unknowingly spread the disease by moving vector and infected hosts.

## SYMPTOMS

The symptoms of Texas Phoenix palm decline (16SrIV-D) and lethal yellowing (16SrIV-A) on date palm appear to be exactly the same, with possibly one exception; root decay has been observed with Texas Phoenix palm decline early in the disease process (Harrison and Elliott, 2012, 2013).

The first obvious phytoplasma disease symptom on mature date palms is premature drop of most or all fruits at one time. The fruit drop occurs within a few days. The fruit drop is not spread out over a prolonged period of time. Inflorescence necrosis follows. However, these two symptoms will only be observed if the palm is mature enough to produce fruit, if it is the season for flowering and fruiting, and if the flowers or fruits have not been trimmed from the palm.

The next symptom is discoloration of the foliage, beginning with the oldest leaves. The leaves do not turn yellow (or do so briefly), but quickly turn varying shades of reddish-brown to dark brown or gray. The discoloration begins at leaf tips. Unless the palm is being monitored closely, the onset of leaf discoloration is usually first recognized as a greater number of dead older leaves than is normal for natural senescence. This symptom might be confused with other problems, such as early senescence due to nutrient deficiency (e.g., potassium) or Ganoderma butt rot caused by *Ganoderma zonatum*, a common fungal disease of landscape palms in Florida.

When less than 30% (and usually less than 25%) of the oldest leaves have discolored and become necrotic, the spear leaf dies. Death of the spear leaf indicates the apical meristem (bud) has died. Once the apical meristem has died, no new leaves will develop, and the leaves remaining in the canopy will continue to discolor from the oldest to the youngest leaves (Fig. 2).

In some instances, by the time the spear leaf dies due to Texas Phoenix palm decline (16SrIV-D), mature roots of the palm at or near the soil surface are soft in texture and easily broken. The palm can be easily rocked back and forth in the ground because the root system is decaying. This symptom is not typical for palms affected by LY (16SrIV-A).

## DIAGNOSTICS

Initial diagnosis of group 16SrIV palm phytoplasmas in the USA is based on field observation of symptoms described above. Since phytoplasmas remain unculturable, DNA-based molecular diagnostic assays are used as the method of choice to confirm the presence of the pathogen in palm tissues. Interior stem tissues provide the most reliable and convenient source of DNA required for analysis by these assays (Cordova et al., 2014; Oropeza et al., 2011). Tissue removal from affected palms is routinely accomplished by drilling a hole into the lower stem (http://flrec.ifas.ufl.edu/pdfs/LY-TPPD-Trunk-Sampling.pdf). The process is easily accomplished using a drill with an auger bit of sufficient diameter and length (~1 cm X 30 cm) to obtain a suitable supply of tissue shavings containing vascular tissue. During sample acquisition it is important that samples are acquired by drilling into the stem to a depth beyond the pseudobark (epidermis plus cortex), which contains no vascular tissue, to reach the underlying interior tissue containing vascular bundles, as the phytoplasma is present only with the phloem of these bundles. Tissue samples should be collected directly into clean, sealable plastic bags, labeled appropriately and kept in a cooler for transportation to the laboratory. It is essential to clean the drill bit if more than one palm is to be sampled. This is best achieved by rinsing the bit free of debris with a stream of water and then flame sterilizing the bit with a portable propane torch to remove any traces of DNA. The bit should be cooled by a second application of water before sampling the next palm.

**Fig 2.** Comparison of healthy date palms with date palms infected with 16SrIV-D phytoplasma on the west-central coast of Florida, USA.

Detection of phytoplasma DNA in stem samples by use of polymerase chain reaction (PCR) assays is the preferred method for confirming phytoplasma diseases of palms. Extraction of total DNA by standard methods for analysis by nested PCR assays has been described in detail (Harrison and Oropeza, 2008; Harrison et al., 2013). Typically 50-100 ng of sample DNA is required as template for analysis. PCR assays incorporating phytoplasma universal *rrn* operon primer pairs are typically used in situations in which a phytoplasma disease is suspected but the identity of the particular strain is not known. Although universal primers amplify rDNA products from all phytoplasmas, products of expected size must be sequenced to confirm their identity as these primers may occasionally amplify rDNA products of similar size from non-target microbes also. Once the identity of the phytoplasma has been established, or is already known, assays employing primer pairs capable of detecting phytoplasmas in a group or subgroup-specific manner can then be used. Confirmation of positive detections may then be identified and compared by RFLP analysis following digestion of PCR products with key restriction endonuclease enzymes (Fig. 3) and/or sequenced, if needed. Real-time PCR assays for universal and group specific detection of phytoplasmas represent a more recent development for confirming phytoplasmas on palms while enhancing detection sensitivity (Cordova et al., 2014; Hodgetts et al., 2009).

In practice, molecular diagnostics are best used to confirm the presence of phytoplasma disease in symptomatic palms in a nursery or community settings in order to track the spread of the disease and to devise a management program for remaining, susceptible palms. However, this molecular testing does not certify that a palm is phytoplasma free.

## DISEASE MANAGEMENT

If the spear leaf has died, the palm should be removed as soon as possible. Death of the spear leaf indicates the apical

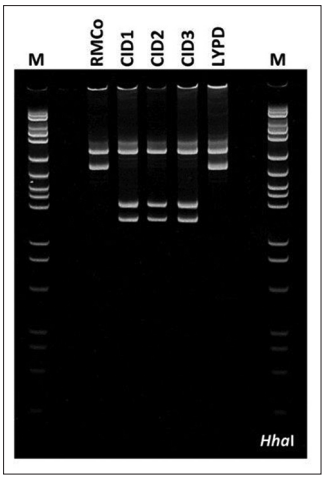


**Fig 3.** In Florida, USA, routine diagnostics includes amplification of phytoplasma rDNA products (1.6 kb) from symptomatic palms by nested PCR with rRNA gene operon primer pairs P1m/LY16–23Sr and then LY16Sf2/LY16–23Sr2. These products are then subjected to endonuclease digestion with *HhaI* to determine which phytoplasma was infecting the palm. Palms RMCo and LYPD illustrate the pattern obtained for 16SrIV-A. Palms CID1-CID3 illustrate the pattern obtained for 16SrIV-D.

meristem (bud) has died, so no new growth will occur. Although lower leaves may remain green for a number of months after the spear leaf dies, it is in the best interest of the nursery grower or the community to remove the infected palm as soon as possible. The diseased palm serves as a source of the phytoplasma that can be transmitted by an insect vector to still-healthy, but susceptible palms (Harrison and Elliott, 2012, 2013).

If symptoms are present, but the spear leaf has not died, therapeutic treatment of the disease may be achieved by application of the antibiotic oxytetracycline HCl, administered to palms by liquid injection into the trunk (McCoy, 1975, 1982). However, the most effective use of the antibiotic is as a preventive treatment to protect palms susceptible to the 16SrIV phytoplasmas when these phytoplasmas are known to occur in the area. Both therapeutic and preventive antibiotic treatments should be made every three to four months. For large palms such as date palms, 3 grams of the antibiotic per palm is recommended. The product is mixed with water and then injected into the palm trunk under low pressure into a pre-drilled port. In the USA, fruit from palms injected with this antibiotic cannot be used for human consumption.

No control of the vector or vectors is recommended in the landscape. However, many palm nurseries do apply insecticides to keep vector populations down, but no research has been conducted to determine the effectiveness. Use of host resistance represents the most practical long-term solution.

## CONCLUSION

Only one major group of phytoplasmas, namely group 16SrIV, has been identified in any palm in the continental USA. Three subgroups (16SrIV-A, D and F) have been detected in *Phoenix dactylifera* (date palm) in Florida, but only subgroup 16SrIV-D has been detected in date palm in Texas. This subgroup has been detected in Louisiana, but thus far only in *Phoenix canariensis*. Florida, Louisiana, Texas and most other southern USA states use date palms for landscape purposes and not for food production. Confirmation of this phytoplasma subgroup in date palm in California, where such palms are used for both landscapes and food production, has been inconclusive. Palm hosts of the 16SrIV subgroups and plant hosts of the insect species that vector the phytoplasmas include more than date palms. Plus, subgroups 16SrIV-A and D occur elsewhere in the Caribbean Basin (Ntushelo et al., 2013). Thus, movement of the phytoplasma (and vector hosts), both naturally and by humans, will likely continue, resulting in expansion of the geographic range of these phytoplasma subgroups.

### Author contribution

All authors contributed equally in this article.

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