‘*Candidatus* Phytoplasma palmae’ and related strains

Scientific Name
‘*Candidatus* Phytoplasma palmae’

Note: The provisional name ‘*Candidatus* Phytoplasma palmae’, based on phylogenetic analysis to denote the phytoplasma associated with lethal yellowing disease of palms in the Americas, is commonly used but has yet to be formally accepted. A formal species description is being prepared for publication (Harrison, 2011).

Common Name(s)
Palm lethal yellowing, lethal yellowing, coconut lethal yellowing, Texas Phoenix decline, Texas Phoenix palm decline, date palm lethal decline, Washingtonia palm decline, Yucatan coconut lethal yellows, coconut lethal disease, coconut lethal decline, *Carludovica palmata* yellows, coyol palm decline, and coconut leaf yellowing.

Type of Pest
Phytoplasma

Taxonomic Position
Class: Mollicutes; **Order:** Acholeplasmatales; **Family:** Incertae sedis; **Genus:** ‘*Candidatus* Phytoplasma’

Reason for Inclusion in Manual
Palm survey; National threat

Pest Description
Phytoplasmas, formerly known as mycoplasma-like organisms (MLOs), are pleomorphic, cell wall-less bacteria with small genomes (530 to 1350 kbp) of low G + C content (23-29 mol%). They belong to the class Mollicutes and are the putative causal agents of yellows diseases that affect at least 1,000 plant species worldwide (McCoy et al., 1989; Seemüller et al., 2002). These minute, endocelluar prokaryotes colonize the phloem of their infected plant hosts as well as various tissues and organs of their respective insect vectors. Phytoplasmas are transmitted to plants during feeding activity by their vectors, primarily leafhoppers, planthoppers, and psyllids (IRPCM, 2004; Weintraub and Beanland, 2006).

Although phytoplasmas cannot be grown by laboratory culture in cell free media, they may be observed in infected plant or insect tissues by use of electron microscopy or detected by molecular assays incorporating antibodies or nucleic acids. Since biological and phenotypic properties in pure culture are unavailable as aids in their identification, phylogenetic analysis of 16S rRNA genes has been adopted instead as a basis for phytoplasma taxonomy. The provisional taxonomic status of ‘*Candidatus*’ used for incompletely described microorganisms has been assigned to the genus (*i.e.,*

'*Candidatus* Phytoplasma') and descriptions of species (*i.e.,* '*Ca*. Phytoplasma species') are underway following established guidelines (IRPCM, 2004; Harrison et al., 2011).

Palms have enormous appeal in many cultures and are valued for their aesthetics. Their fruit, seeds, leaves (fronds), and stems are highly valued and are used for a variety of purposes from food to biofuels to cosmetic production and timber (Gitau et al., 2009). The descriptive term lethal yellowing (LY), was first used by Nutman and Roberts (1955) to denote a fast spreading, fatal disease of coconut palm in Jamaica. LY has since destroyed millions of coconut and other palm species on various Caribbean islands (Bahamas, Cuba, Hispaniola, and Jamaica), the mainland United States of America, as well as in Mexico, Belize and Honduras (Eden-Green, 1997).

Figure 1: Leaf yellowing on *Cocos nucifera*. Photo Courtesy of Dr. Nigel Harrison, University of Florida, Dr. Monica Elliott, University of Florida, Institute of Food and Agricultural Sciences, and Ian Maguire, University of Florida, Institute of Food and Agricultural Sciences.

Symptoms matching those of lethal yellowing were first described on coconut palms in the Cayman Islands in 1834. The disease then spread to other islands throughout the Caribbean region including Haiti, Dominican Republic, Cuba, Jamaica, and then Florida (McCoy et al., 1983; Dollet et al., 2009; Gitau et al., 2009; Eziashi and Omamor, 2010). The disease reached the Yucatan Peninsula of Mexico during the 1980s and was reported in Honduras in 1996 (Ashburner et al., 1996), Guatemala in 2004 (Mejía et al., 2004), and the northern Leeward Islands by 2006 (Myrie et al., 2006). There were also reports of a similar disease affecting Phoenix palms near Brownsville in the Rio Grande valley of Texas during the 1970s (McCoy et al., 1980).

Detection and characterization of phytoplasmas relies primarily upon Restriction Fragment Length Polymorphism (RFLP) typing of 16S rRNA gene sequences (1.2 kb) amplified by Polymerase Chain Reaction (PCR) employing phytoplasma 'universal' primer pairs (Gundersen and Lee, 1996; Lee et al., 2000). This approach has also provided a means to classify phytoplasmas into a series of distinct 16Sr groups and subgroups of strains (Lee et al., 1998; Zhao et al., 2009). By this approach, the phytoplasma associated with LY disease *sensu* (Nutman and Roberts,1955) was assigned to group 16SrIV (coconut lethal yellows group) as a subgroup A (*i.e.,* 16SrIV-A) member (Lee et al., 2000). Within group 16SrIV, five other related phytoplasmas have since been classified into additional subgroups, namely, 16SrIV-B, 16SrIV-C, 16SrIV-D, 16SrIV-E and 16SrIV-F (Lee et al., 2000; Wei et al., 2007; Harrison et al., 2008). Group 16SrIV phytoplasmas limited to the Americas will be covered in this pest datasheet (Table 1).

Groups resolved by RFLP typing largely conform to subclades (*i.e.,* primary groups) of phytoplasmas delineated by phylogeny of near full-length 16S rRNA gene sequences upon which a formal taxonomy of phytoplasmas is now based. '*Candidatus* Phytoplasma species' names are being assigned to a reference strain within each primary group (IRPCM, 2004; Harrison et al., 2011). Both systems may be used separately or in combination to identify phytoplasmas. Currently, the proposed reference strain for '*Ca*. Phytoplasma palmae' (IRPCM, 2004) corresponds to subgroup 16SrIV-A by RFLP typing, and members of other 16SrIV subgroups are, by convention, referred to as '*Ca*. Phytoplasma palmae'-related strains.

Lethal yellowing has also been used to describe phytoplasma-associated diseases of coconut palm that reportedly occur at several locations throughout the Old World humid tropics. Despite overall similarities in symptomatology between these diseases and LY, epidemiological considerations such as geographical distribution patterns, rates of spread, and varietal and host species susceptibility have indicated dissimilarities among causal agents and vector species involved with these diseases. To acknowledge these differences they are collectively referred to as "LY-type" diseases (Eden-Green, 1997; Dollet et al., 2009).

At least five additional phytoplasmas, *'Candidatus* Phytoplasma cocostanzaniae' (Group 16SrIV-C), '*Ca*. Phytoplasma cocosnigeriae' (Group 16SrXXII), '*Ca*. P. cynodontis' (16SrXIV), '*Ca*. Phytoplasma oryzae' and '*Ca*. Phytoplasma malaysianum' are associated with diseases of palms (Table 2). Each is phylogenetically distinct from '*Ca*. Phytoplasma palmae' and will not be covered in detail in this datasheet (Table 2). These diseases are often known by a variety of names depending upon location. They include, Cape St. Paul wilt in Ghana, Kaincopé in Togo, bronze leaf or Awka wilt in Nigeria, Kribi in Cameroon, lethal disease in Tanzania and Kenya (Eden-Green 1997), lethal yellowing in Mozambique (Bonnot et al., 2010), Kalimantan wilt in Indonesia, (Warokka et al., 2006), coconut yellow decline in Malaysia (Nejat et al., 2009a,b), coconut (root) wilt (Manimekalai et al., 2010) and yellow leaf disease of *Areca catechu* (betel nut) (Ramaswamy et al., 2012) in India, Weligma coconut leaf wilt in Sri Lanka (Perera et al., 2012), and Bogia coconut syndrome in Papua New Guinea (Kelly et al., 2011).

Table 1: '*Candidatus* Phytoplasma palmae' and related strains organized by name and location.

Strain	Disease Common name	16S rDNA group/subgroup[1]	Known Distribution[2]
A	Palm lethal yellowing	Group 16SrIV, subgroup A (16SrIV-A)	BZ, KY, CU, DO, GT, HT, HN, JM, MX, KN, US (Myrie et al., 2006; Harrison and Oropeza, 2008; Harrison, 2012b)
	Coconut lethal yellowing		US (FL) (Harrison et al., 2002a)

B	Yucatan coconut lethal decline (YLD) Coyol palm decline	Group 16SrIV, subgroup B (16SrIV-B)	MX (Yucatan Peninsula) (Lee et al., 2002c) HN (Roca et al., 2006)
C	Coconut lethal disease	Group 16SrIV, subgroup C (16SrIV-C)	KE, TZ[3] (Tymon et al., 1998)
D	*Carludovica palmata* yellows, *Sabal mexicana*, and *Pseudophoenix sargentii* decline Texas Phoenix decline, Texas Phoenix palm decline, date palm lethal decline	Group 16SrIV, subgroup D (16SrIV-D)	MX (Cordova et al., 2000; (Vázquez-Euán et al., 2011) US (TX, FL, LA, PR) (Harrison et al., 2002b; Elliott, 2009; Ong and McBride, 2009; Rodriguez et al., 2010; Rodriguez et al., 2011; Harrison, 2012a ; Singh, 2014)
E	Coconut lethal decline N/A	Group 16SrIV, subgroup E (16SrIV-E)	DO (Martinez et al., 2008) JM (Brown and McLaughlin, 2011)
F	*Washingtonia robusta* decline	Group 16SrIV, subgroup F (16SrIV-F)	US (FL) (Harrison et al., 2008)

[1] The classification based on 16S rDNA restriction fragment length polymorphism (RFLP) analysis.
[2]BZ Belize; CU Cuba; DO Dominican Republic; FL Florida, GT Guatemala; HN Honduras; HT Haiti; JM Jamaica; KN Saint Kitts and Nevis; KY Cayman Islands; MX Mexico; PR Puerto Rico; US United States.
[3] Strain C is also informally referred to as '*Candidatus* Phytoplasma cocostanzaniae'.

Table 2: Other '*Candidatus* Phytoplasma' species associated with lethal yellowing-type diseases of palms.

Species	Disease Common name	16 S rDNA group/subgroup[1]	Known Distribution[2]
'Candidatus Phytoplasma cocosnigeriae'	Nigerian Awka wilt disease Coconut lethal yellowing	Group 16SrXXII, subgroup A (16SrXXII-A)	NG (Tymon et al., 1998; IRPCM, 2004) MZ, TG, CM, BJ, GQ (Bonnot et al., 2010)

	Cape St. Paul wilt	Group 16SrXXII, (unclassified subgroup)	GH (Tymon et al., 1998)
'Candidatus Phytoplasma cocostanzaniae'	Coconut lethal disease	Group 16SrIV-C	KE, TZ (IRPCM, 2004)
'*Candidatus* Phytoplasma cynodontis'	Bermudagrass white leaf; white tip dieback, slow decline	Group 16SrXIV (unclassified subgroup)	MY, SD (Cronje et al., 2000a; b; Nejat et al., 2009b)
'*Candidatus* Phytoplasma malaysianum'	Coconut yellow decline (MYD) Malayan oil palm disease (MOP)	Group16SrXXXII, subgroup 16SrXXXII-B) (subgroup 16SrXXXIII-C)	MY (Nejat et al., 2009a; 2012)
'*Candidatus Phytoplasma oryzae'*	Yellow leaf disease of areca palm Coconut (root) wilt	Group 16SrXI (subgroup 16SrXI-B) (unclassified subgroup)	IN (Ramaswamy et al., 2012) (Manimekalai et al., 2010)

[1] Classification based on 16S rDNA restriction fragment length polymorphism (RFLP) analysis.
[2] BJ Benin; CM Cameroon; GH Ghana GQ, Equatorial Guinea; IN India; KE Kenya; MY Malaysia; MZ Mozambique; NG Nigeria; SD Sudan; TG Togo; TZ Tanzania.

Biology and Ecology

'*Candidatus* Phytoplasma palmae' is most readily observed in immature tissues of palms by electron microscopy (Thomas,1979; Thomas and Norris, 1980) and reliably detected in immature palm host tissues by PCR assays employing phytoplasma 'universal' and/or group-specific rRNA operon primer pairs (Cordova et al., 2003; Harrison and Oropeza, 2008; Harrison et al., 2008). Although '*Ca*. Phytoplasma palmae' DNA has been detected in embryos from fruit of infected coconut palm (Cordova et al., 2003), there is no evidence that this pathogen is transmitted through seed.

Phytoplasmas are transmitted to plants in a circulative-propagative manner by phloem-feeding insect vectors. Their ingestion of sap from diseased plants is followed by an incubation phase lasting for one to several weeks during which time these bacteria circulate, multiply, and parasitize various tissues and organs of their respective vectors. Once salivary glands have been colonized, vectors are then capable of transmitting phytoplasmas during any subsequent feeding activity for their remaining lifespan (Weintraub and Beanland, 2006; Gitau et al., 2009).

The only known vector of '*Ca*. Phytoplasma palmae' is the neotropical planthopper *Haplaxius* (syn. *Myndus*) *crudus* (American palm cixiid) (Howard et al., 1983; Eziashi and Omamor, 2010). *H. crudus* is found in North, Central, and South America, and also

in the Caribbean region. In areas affected by lethal yellowing, *H. crudus* was 40 times more abundant than in areas where the disease does not occur (EPPO, n.d.). Purcell (1985) noted that *H. crudus* is a very inefficient vector, but it is so abundant that a very low transmission rate is sufficient to spread LY disease.

Figure 2: Coconut inflorescence necrosis an early symptom of lethal yellowing. Photo courtesy of Dr. Nigel Harrison, University of Florida.

Primary infection of the highly susceptible Atlantic tall coconut ecotype is followed by a prolonged latent (symptomless) phase, estimated between 112 to 262 days in young nonbearing palms to as long as 450 days in mature palms of large stature (Dabek, 1975).

Systemic treatment of susceptible palm species with oxytetracycline-HCl (OTC) administered by stem injection at four month intervals results in a remission of early stage symptoms. OTC treatment is also very effective in preventing disease from occurring on healthy palms (Hunt et al., 1974; McCoy, 1982). However, such treatments are not economically practical as a disease management strategy except for palms valued as ornamentals in landscape and amenity plantings. Host resistance, utilizing LY-resistant palm species has been the primary disease management strategy employed against LY.

Compared to other palm species, coconut is considered to be most susceptible to lethal yellowing. Among coconut ecotypes and hybrids, Malayan dwarf and Maypans were once considered to be highly resistant to LY. However, unusually high mortality of both cultivars in some LY-affected areas in recent years indicates that neither can be considered resistant (Broschat et al., 2002; Lebrun et al, 2008). Environmental and genotype x environmental factors, however, can exert a significant influence on the overall performance of coconut cultivars in LY endemic areas (Ashburner and Been, 1997; Zizumbo et al., 1999).

Symptoms and Signs

Before visible symptoms are first observed, coconut palms infected by '*Ca.* Phytoplasma palmae' undergo a range of measurable biochemical and physiological changes. About 80 days prior to the appearance of symptoms, growth of affected palms is measurably stimulated. A period of gradual decline followed by complete inhibition of growth then occurs about one month before the end of the symptomless phase. These changes are also accompanied by decreased respiration and increased root necrosis (Harrison and Oropeza, 2008).

No single symptom associated with lethal yellowing is diagnostic of the disease. Each symptom may vary according to the particular species and cultivar of palm affected and by a variety of other causes. Rather it is the sequential and progressive development of symptoms (syndrome) that identify LY and help distinguish it from other diseases and disorders that induce similar but isolated symptoms.

For mature, bearing coconut and other mature palm species infected by '*Ca*. Phytoplasma palmae', the earliest visible symptom is a premature shedding of most or all fruit regardless of developmental stage. Fruit that are shed from coconut often develop a blackened, or water soaked appearance at the calyx end. Necrosis of newly emergent inflorescences accompanies or follows fruit drop. Flower spikelets, which are normally light yellow to creamy white in color, appear partially or totally blackened. Fruit and flower symptoms are followed shortly thereafter by foliar discoloration. On the Atlantic tall coconut ecotype, leaves turn a golden yellow color (Fig. 1). Discoloration begins on the lowermost (oldest) leaves and progresses to successively younger leaves in the upper part of the crown. Discolored leaves typically remain turgid for some time before turning brown, drying and hanging downward around the stem for a few days before falling to the ground. The newest unopened leaf (spear) collapses, once foliar discoloration is advanced. Death of the apical meristem occurs at this stage after which the remaining crown withers and topples away leaving just a bare trunk standing.

While premature fruit drop and inflorescence necrosis (Fig. 2) are common to all palms with lethal yellowing, leaves turn reddish brown rather than yellow on many coconut ecotypes and most other palm species (Fig. 3). On date palms, death of the spear leaf and underlying apical meristem occurs shortly after leaves first begin to discolor (Harrison and Jones, 2004; Downer, 2009; Broschat et al., 2010). Most affected palms die within 3 to 5 months after the onset of symptoms (McCoy et al., 1983; Broschat, et al., 2010; Eziashi and Omamor, 2010).

For preliminary field diagnosis of disease, symptoms on palms induced by '*Ca*. Phytoplasma palmae'-related strains (i.e. subgroups 16SrIV-B, 16SrIV-D, 16SrIV-E and 16SrIV-F strains) are not sufficiently distinct in appearance to distinguish them from those attributed to '*Ca*. Phytoplasma palmae'. For example, on *Phoenix sylvestris*, symptoms of Texas Phoenix palm decline (TPPD) attributed to subgroup 16SrIV-D phytoplasmas, foliar discoloration begins on the lowermost (oldest leaves), which turn reddish brown, starting at the tips and intensifies to involve successfully younger leaves in the mid-crown and upper crown (Fig. 4). Shedding of most or all fruits as inflorescences wither and die prematurely is accompanied by collapse and death of the newest (spear) early in the foliar discoloration phase. Once discoloration has progressed to leaves of the mid-crown, mature roots of palms at or near the soil are unusually soft in texture and easily severed. Palms that have undergone these adverse changes can be easily pushed back and forth. However, loss of the structural integrity of the root system has not been demonstrated for any other palm species affected by subgroup 16SrIV-D phytoplasmas (Harrison et al., 2008).

Figure 3: These are all symptoms of palms infected with palm lethal yellowing strain A. The top left photo shows the spear leaf of this *Phoenix sylvestris* has collapsed and is hanging down (right side of the trunk).The top right photo shows lethal yellowing *Cocos nucifera* in various stages. Healthy plants in the back. Palms in the front are in the early-mid stages. Palms to the left and right are dead and the trunk bare. The bottom left photo shows different leaf shades on *Phoenix canariensis*. The bottom right photo shows symptoms of leaf color of reddish brown on Maypan dwarf cultivar of *Cocos nucifera*. Photo Courtesy of Dr. Nigel Harrison, University of Florida, Dr. Monica Elliott, University of Florida, Institute of Food and Agricultural Sciences, and Ian Maguire, University of Florida, Institute of Food and Agricultural Sciences.

Figure 4: These are all symptoms of palms infected with Texas Phoenix Palm Decline Strain D. The top left photo shows initial symptoms of lethal yellowing in *Phoenix sylvestris*. The early leaves are yellowing then eventually the leaves completely die. The top right shows early fruit fall in *P*. *dactylifera*. The bottom left is a photo of *P*. *sylvestris* showing the reddish-brown color that happens during foliar discoloration also this palm exhibits a significant leaf lost. The bottom right shows leaf dead and death of the spear leaf in *Sabal palmetto*, notice the young leaves are still green. Photo Courtesy of Dr. Nigel Harrison, University of Florida, Dr. Monica Elliott, University of Florida, Institute of Food and Agricultural Sciences, B. Dick and Ian Maguire, University of Florida, Institute of Food and Agricultural Sciences.

Pest Importance

'*Candidatus* Phytoplasma palmae' is known to have significant impacts on palm production. Because of the capacity for rapid spread and high susceptibility of coconut populations in most regions where LY occurs, the disease is considered to be one of the most important global threats to coconut production (Eden-Green, 1997). For example, since 1971, the original population of Atlantic tall coconut ecotype, once estimated at 700,000 palms, has been largely destroyed by LY in southern Florida. Catastrophic losses, estimated at 7 million palms, of this highly susceptible ecotype also occurred in Jamaica during the same era and were followed by similar large scale mortality of coconut to LY along the Atlantic coasts of Mexico and Honduras. Such epiphytotics have a huge economic impact on countries whose rural economies rely upon coconut production as sources of food, oil, biofuels, timber, wine, charcoal, etc. or for sale as landscape ornamentals (Gitau et al., 2009).

Known Hosts

Subgroup 16SrIV-A strains: *Adonidia* (*Veitchia*) *merrillii* (Christmas or Manila palm), *Aiphanes lindeniana* (ruffle palm), *Allagoptera arenaria* (Kuntze seashore palm), *Arenga engleri* (dwarf sugar palm), *Borassus flabellifer* (palmyra palm), *Bismarkia* sp. (Bismark palm), *Caryota mitis* (Burmese or clustering fishtail palm), *Caryota rumphiana* (giant fishtail palm), *Chelyocarpus chuco* (round leaf palm), *Cocos nucifera* (coconut palm), *Copernicia alba* (Caranday palm), *Corypha taliera* (buri palm), *Crysophila warsecewiczii* (rootspine palm), *Cyphophoenix nucele* (lifou palm), *Dictyosperma album* (princess or hurricane palm), *Dypsis cabadae* (cabada palm), *Dypsis decaryi* (triangle palm), *Gaussia attenuata* (Puerto Rican gaussia palm), *Howia belmoreana* (belmore sentry palm), *Howea forsteriana* (Kentia or sentry palm), *Hyophorbe verschaffeltii* or *Mascarena verschaffeltii* (spindle palm), *Latania lontaroides* (latan palm), *Livistona chinensis* (Chinese fan palm), *Livistona rotundifolia* (footstool palm), *Nannorrhops ritchiana* (mazari palm), *Phoenix canariensis* (Canary Island date palm), *Phoenix dactylifera* (date palm), *Phoenix reclinata* (Senegal date palm), *Phoenix rupicola* (cliff date palm), *Phoenix sylvestris* (silver date palm), *Pritchardia affinis* (Kona palm), *Pritchardia pacifica* (Fiji island fan palm), *Pritchardia remota* (Remota loulu palm), *Pritchardia thurstonii* (Thurston palm), *Ravenea hildebrantii* (Hildebrandt's palm), *Roystonia regia* (royal palm), *Syagrus schizophylla* (arikury palm), *Trachycarpus fortunei* (windmill palm), *Veitchia arecina*, *Veitchia mcdanielsi* (sunshine palm), *Veitchia montgomeryana* (Montgomery's palm), and *Wodyetia bifurcata* (foxtail palm) (McCoy et al., 1983; Eden-Green, 1997; Harrison and Jones, 2004; Harrison and Oropeza, 2008; Myrie et al., 2014; EPPO, n.d).

Non-palm hosts: Common screwpine (*Pandanus utilis*: Pandanaceae) (Thomas and Donselman, 1979).

Symptomless Hosts: *Thrinax radiata* (Florida thatch palm) and *Coccothrinax readii* (Mexican silver palm) (Narvaez et al., 2006).

Subgroup 16SrIV-B strains: *Acrocomia aculeata* (coyol palm) and *Cocos nucifera* (coconut palm) (Harrison et al., 2002c; Roca et al., 2006).

Subgroup 16SrIV-D strains: *Caryota urens* (jiggery or toddy palm), *Phoenix canariensis* (Canary Island date palm), *Phoenix dactylifera* (date palm), *Phoenix reclinata* (Senegal date palm), *Phoenix roebelenii* (pygmy data palm), *Phoenix sylvestris* (silver date palm), *Roystonea* sp., *Sabal palmetto* (sabal or cabbage palm), *Sabal mexicana* (Mexican palmetto; Texas palmetto; Rio Grande palmetto), *Syagrus romanzoffiana* (queen palm); *Syagrus romanzoffiana* x *Butia capitata* (mule palm), and *Washingtonia robusta* (Washington fan palm) (Harrison et al., 2002b; Elliott, 2009; Harrison et al., 2008; 2009; Aviña-Padilla et al., 2011; Vázquez-Euán et al; 2011).

Non-palm hosts: Panama hat palm (*Carludovica palmata*: Cyclanthaceae) (Cordova et al., 2000).

Subgroup 16SrIV-E strains: *Cocos nucifera* (coconut palm).

Non-palm hosts: *Cleome rutidosperma* (fringed spiderflower), *Cyanthillium cinereum* (little ironweed cited as *Vernonia cinerea*), *Macroptilium lathyroides* (wild bushbean), and *Stachytarpheta jamaicensis* (light-blue snakeweed) (Brown et al., 2008; Martinez et al., 2008; Brown and McLaughlin, 2011).

Subgroup 16SrIV-F strains: *Phoenix dactylifera* (date palm) and *Washingtonia robusta* (Washington fan palm) (Harrison et al., 2008).

Figure 5: Male (left) and Female (right), *Haplaxius* (*Myndus crudus*), the vector of *Ca*. Phytoplasma palmae. Photo courtesy of Dr. Nigel Harris, University of Florida.

Known Vectors or Associated Insects

'*Candidatus* Phytoplasma palmae' is known to be vectored by the planthopper *Haplaxius* (syn. *Myndus*) *crudus* (Fig. 5). Other *Haplaxius* sp. and *Cedusa* sp. are suspected vectors of LY (Brown et al., 2006).

Known Distribution

The following '*Candidatus* Phytoplasma palmae'-related strains are limited in distribution to the Americas although the identity of strains associated with historical examples of disease prior to the availability of molecular diagnostic methods is not known.

Subgroup 16SrIV-A: **Caribbean:** Antigua, Bahamas, Cayman Islands, Cuba, Dominican Republic, Haiti, Jamaica, Saint Kitts and Nevis, Antigua. **Central America:** Belize, Honduras, and Guatemala. **North America:** Mexico and United States (Florida); Harrison et al., 2002a; Myrie et al., 2006; Myrie et al., 2014).

Subgroup 16SrIV-B: **Central America:** Honduras (Roca et al., 2006). **North America:** Mexico.

Subgroup 16SrIV-D: **Caribbean:** Puerto Rico. **North America:** Mexico and United States (Florida, Louisiana, Texas) (Cordova et al., 2000; Harrison et al., 2002b; Elliott, 2009; 2009; Rodriguez et al., 2010; 2011; Vázquez-Euán et al., 2011; Harrison, 2012a; Singh, 2014).

Subgroup 16SrIV-E: **Caribbean:** Dominican Republic and Jamaica (Martinez et al., 2008; Brown et al., 2006).

Subgroup 16SrIV-F: **North America:** United States (Florida) (Harrison et al., 2008).

Pathway

The pathway of transmission is via the planthopper vector *H. crudus*. Phytoplasmas can also spread from infected propagative plant material. Although phytoplasma DNA has been detected in embryos from some fruit of diseased coconut palms (Cordova et al., 2003), there is no evidence to support seed as a pathway of disease transmission.

Potential Distribution Within the United States

Lethal yellowing of palms attributed to subgroup 16SrIV-A phytoplasmas was first reported in Key West, Florida in 1955 and then in Miami on mainland southern Florida in 1971 (McCoy et al., 1983). Today, the disease is endemic throughout most of South Florida and has spread northward to Sarasota county on the west coast and Indian River county on the east coast in recent years.

Subgroup 16SrIV-D and subgroup 16SrIV-F phytoplasmas were first identified in Florida palms during 2007. Both subgroups are resident in Sarasota, Manatee, and Hillsborough counties of west-central Florida. Isolated cases of disease attributed to subgroup 16SrIV-D strains have been confirmed as far northward as Duval county and in Palm Beach in the southeastern part of the state. Subgroup 16SrIV-D strains occur in Texas where they are limited to coastal areas (Harrison et al., 2002b, Ong and McBride, 2009; NPAG, 2011).

Taken together with reports of this pathogen affecting varieties of coconut palms previously considered highly resistant to lethal yellowing and the possibility of new vectors indicates that this pathogen is a threat to susceptible palms growing in other regions of the United States, such as commercial date palms (*Phoenix dactylifera*) in California and Arizona, popular ornamental palms such as Canary Island date palm (*Phoenix canariensis*), pygmy date palm (*Phoenix roebelenii*) and native palms including cabbage palm (*Sabal palmetto*) throughout the southeastern United States and Rio Grande palmetto (*Sabal mexicana*), a species limited to coastal southern Texas (Howard, 1983; NPAG, 2011).

Survey

CAPS-Approved Method*: The CAPS-Approved method for '*Ca*. Phytoplasma palmae' and related strains is visual survey for symptoms. Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014 and as summarized below.

Palm samples from immature field-grown palms with symptoms suggestive of phytoplasma disease should be received as freshly harvested leaflets (pinnate species) or leaflet lamina and midvein tissues (palmate species) taken from the youngest leaf (*i.e.,* spear).

For mature palms, tissue samples can be removed as stem borings.

- Prior to sampling each palm, the bit should be flame sterilized using a portable propane torch and cooled by rinsing with water.
- Stem samples are removed by boring a hole (10 to 15 cm in length) into the palm stem (trunk) using a portable electric drill and 5/16 in. (ca. 7.8 mm) diameter bit.
    - Begin sampling by drilling a shallow pilot hole in the lower stem to remove the outermost layer of pseudobark (discard these tissues).
    - Resume drilling incrementally through the pilot hole into the interior stem to the final depth of ~15 cm using a back and forth motion to dislodge shavings.
- Tissue borings from the stem are collected directly into a clean sealable plastic bag.
- Once the sampling is complete, the stem can be sealed (if necessary) by tapping a wooden dowel into the hole to prevent sap bleeding and to provide a barrier to invasion by pests (see Harrison et al., 2013 for more details).

Each freshly harvested sample will be packaged separately, labeled, documented and shipped for laboratory testing by overnight express service according to APHIS regulations. Each sample, in a separate plastic bag, will be labeled according to cultivar (when known) and plant species, date sample was collected, location of plant sampled, and name and institution of sample collector; each plastic bag should contain samples collected from only a single plant. For extended transport, stem tissues will be shipped after first drying each sample at 37°C (99°F).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Key Diagnostics/Identification

CAPS-Approved Method*: Follow instructions in Phytoplasma sample submission for Cooperative Agricultural Pest Survey (CAPS) Program and Farm Bill Goal 1 surveys FY 2014.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Culture: The phytoplasma that causes lethal yellowing is an obligate parasite and cannot be cultured on microbiological growth media.

Electron Microscopy: Phloem restricted phytoplasmas can be detected by transmission electron microscopy (Thomas and Norris, 1980). In coconut, nonfilamentous forms average 295 nm in diameter and filamentous forms average 142 nm in diameter and at least 16 µm in length (Waters and Hunt, 1980).

Fluorescence Microscopy: For large scale diagnosis, the DAPI (4', 6'-diamidino-2-phenilindole, 2HCl) staining method (Andrade and Arismendi, 2013) can be used, although the percentage of false negative detections can reach high levels, especially in palms. False negatives generally occur when phytoplasma colonization of plants is poor or uneven. This test detects fluorescence of DNA-containing phytoplasma cells in the sieve tubes of the leaf veins.

Transmission electron microscopy and fluorescence microscopy only reveal the presence of phytoplasma. Neither is specific to which phytoplasma might be affecting the host (Harrison et al., 1999).

Molecular:

DNA dot hybridization assays have been used to detect the lethal yellowing phytoplasma by using random fragments of phytoplasma genomic DNA cloned from LY-diseased Manila palm (*Adonidia* (*Veitchia*) *merrillii*) or windmill palm (*Trachycarpus fortunei*) as probes (Harrison et al., 1992; Harrison and Oropeza, 2008). These probes, however, have been shown to vary in detection sensitivity and specificity (Harrison et al., 2008).

Southern blot hybridization analysis of phytoplasma DNA restriction profiles with cloned probes has been used to provide a measure of genetic variability among closely related phytoplasma strains (Harrison et al., 1992; 2008).

A 'universal' PCR assay has been developed that enables amplification of the 16S rRNA genes of phytoplasmas. These assays readily amplify rDNA of most, or all,

phytoplasmas. Digestion of the PCR products with selected restriction enzymes, a process known as restriction fragment length polymorphism (RFLP), provides a DNA fingerprint in the form of 16S rDNA fragment patterns that can be used to determine phytoplasma identity when resolved on agarose or by polyacrylamide gel electrophoresis (PAGE). These primers, however, have also identified non-phytoplasma target sequences. The latter PCR products are similar in size to PCR products from phytoplasmas, so the phytoplasma identity is not known (Harrison et al., 1999). Profiles resolved by PAGE after separate digestion of products with *Alu*I, *Hin*fI, *Taq*I or *Tru*9I endonucleases are especially useful for identification of group 16SrIV phytoplasmas (Harrison et al., 1999).

Group or subgroup-specific detection of phytoplasmas by utilizing primers for PCR based upon variable regions of the 16S rRNA gene or the 16-23S intergenic spacer region (SR) sequences of the phytoplasma genome reportedly permit selective amplification of rRNA gene sequences of '*Ca*. Phytoplasma palmae' and related strains in a group-specific manner. Primers 503f and LY16Sr derived from the 16S rRNA gene of the LY phytoplasma selectively amplify a 928 bp rDNA product from the LY phytoplasma strains infecting coconut and *Pandanus* and from the YLD (Yucatan coconut lethal decline) and CPY (*Carludovica palmata* yellows) phytoplasmas (Harrison et al., 1999; Cordova et al., 2000). Strains can be further differentiated by *Alu*I digestion of the resulting amplification products.

LY16Sf and LY16Sr also selectively amplify 16SrRNA gene sequences of the LY agent from mixtures with host palm DNA (Harrison and Oropeza, 2008). When used to reamplify products obtained by PCR employing universal primer pair P1 and P7, LY16Sf/LY16Sr amplifies of rDNA from the LY phytoplasma and related strains in a group (16SrIV)-specific manner (Harrison et al., 2002a). Polymorphisms revealed by *Hin*fI endonuclease digestion of the rDNA products differentiated coconut-infecting phytoplasmas in Jamaica from those detected in Florida, Honduras, and Mexico (Harrison et al., 2002a).

Exclusive detection of 16SrIV-A subgroup strains is possible by a PCR assay employing nonribosomal primer pair LYF1/LYR1 permitting unequivocal identification of '*Ca*. Phytoplasma palmae' (i.e. subgroup 16SrIV-A) in palms, *Pandanus utilis*, and the vector *Haplaxius crudus* (Harrison et al., 1994; Llauger et al., 2002).

Analysis of less conserved *sec*A gene sequences has also been used to distinguish species and subgroups of phytoplasmas (Hodgetts et al., 2008).

A real-time PCR to detect group 16SrIV subgroups A, D and E found in the Americas will soon be available (Córdova et al., 2014).

Easily Confused Pests

Lethal yellowing symptoms can be confused with those caused by Ganoderma butt rot, boron deficiency, and potassium deficiency on palms. Ganoderma butt rot, caused by the fungus *G. zonatum*, is a basal stem rot that leads to canopy wilting, early death of

lower leaves, and spear leaf. Potassium deficiency will lead to discoloration and early death of the lowest leaves in the canopy. Boron deficiency will lead to early nut fall in coconut. These nuts will not be discolored, nor will they have water-soaked appearance at the calyx of the nut (Broschat, et al., 2010).

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Update History:
June 2013: Original datasheet developed.

June 2014: Louisiana added to distribution section for *Ca.* P. palmae subgroup D. Add three additional hosts for *Ca.* P. palmae subgroup A: *Bismarkia* sp., *Roystonea regia*, and *Wodyetia bifurcata*. Added information about a newly available real-time PCR method to detect *Ca*. P. palmae subgroups A, D, and E in the Americas.