Chapter 9
Dealing with Lethal Yellowing and Related Diseases in Coconut

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9.1 Introduction

The coconut is a plant species of economic and social importance that is cultivated in more than 90 countries around the world. There is a great variety of products that can be obtained from this palm. Their markets, particularly for coconut water, coconut milk, virgin coconut oil and coconut sugar, have been growing exponentially within the past decade (Prades et al. 2016). This represents a very promising future for coconut cultivation and the whole industry. Unfortunately, this growth is threatened by a reduction in fruit production. This is because most palms are now senile and declining in production. In addition, phytoplasma-associated diseases such as lethal yellowing (LY) and similar LY-type diseases (LYDs) have been devastatingly affecting coconut palms in countries of Latin America and the Caribbean (LAC), and Africa (Gurr et al. 2016; Ntushello et al. 2013). These incurable diseases have destroyed millions of coconut palms, as well as the livelihoods of the affected farmers. Thus, it is crucial to develop means and strategies to combat these diseases. This chapter reviews different aspects and approaches that are of importance to management or control.

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9.2 Palm Phytoplasma Diseases in the World

Sightings of coconut palms dying with symptoms like those of LY have been reported since the nineteenth century in Jamaica, Cuba and the Cayman Islands (Ntushello et al. 2013). The disease then spread to other Caribbean Island countries, reaching the continental land, the USA (Florida) and Mexico within the second half of the twentieth century, subsequently moving further south to Honduras (Table 9.1 and Fig. 9.1). In the Caribbean, LY has spread rapidly, entering Antigua by 2010 (Myrie et al. 2014). The outbreak of LY has resulted in the death of millions of coconut palms and corresponding damage to coconut cultivation in LAC. It is interesting that this extensive death of palms has occurred on the Atlantic Ocean side or the east of the Americas, where most coconut were of the Atlantic Tall variety (also known as Jamaican Tall or Brazilian Tall depending on the country), which has been the most extensively cultivated variety. Unfortunately, it happened to be the most susceptible variety to LY. In contrast, LY has been basically absent on the Pacific Ocean or west side of the Americas, where there is a greater diversity of coconut germplasm and the Atlantic Tall variety is not cultivated.

These differences in germplasm between Atlantic and Pacific regions are related to the origin of the introductions of coconut. In the case of Mexico, the Atlantic Tall was originally introduced only to ports on the Atlantic side of Mexico from Cape Verde in Africa, via Puerto Rico ca. 1549 (Zizumbo-Villarreal 1996). In the case of the Pacific coast, other coconut germplasm was introduced from Panama (ca. 1539), Solomon Islands (ca. 1569) and Philippines (between 1571 and 1816) (see Zizumbo-Villarreal 1996). This resulted in a single variety introduced to the east and a great diversity introduced to the west of Mexico and this was the same for the Americas.

LYDs affecting coconut have also been reported in other parts of the world (Table 9.1 and Fig. 9.1). In Africa, LYDs were first observed in Nigeria (West Africa) in the early half of the twentieth century and in Tanzania (East Africa) in the latter half of the twentieth century (Yankey et al. 2018). Other countries in Africa have been also affected. Noticeably, recent outbreaks in Mozambique have killed millions of coconuts while threatening thousands of hectares in Ivory Coast (Yankey et al. 2018) and the COGENT (Coconut Genetic Resources for Enhanced Livelihoods) International Coconut Genebank in Abidjan. Instances of LYDs have also been reported in member countries of the International Coconut Community (ICC) (see Gurr et al. 2016). Occurrences of LYDs affecting other palm species have also been reported in countries in different continents (Table 9.1).

9.3 LY Symptoms

The first visual symptom of LY infection in coconut-bearing palms is the premature drop of most of the fruit regardless of their developmental stage (Fig. 9.2a), followed by the blackening of new inflorescences (Fig. 9.2b and c). This symptom is

Table 9.1 Diversity of phytoplasmas associated with lethal yellowing-type diseases of palm and non-palm species

Species	16SrDNA group-subgroup	Country	Species-disease name	Author
Ca. *Phytoplasma palmae*	Presumably 16SrIV-A[a]	Cuba	Lethal yellowing	Llauger et al. (2002)
		Belize		Escamilla et al. (1994)
		Honduras		Ashburner et al. (1996)
		Guatemala		Mejía et al. (2004)
		Haiti		No confirmed report
		Cayman Islands		No confirmed report
	16SrIV-A	Jamaica	Lethal yellowing	Harrison et al. (2002a)
		Florida (USA)		Harrison et al. (2002a)
		Nevis		Myrie et al. (2006)
		Saint Kitts		Myrie et al. (2012)
		Antigua		Myrie et al. (2014)
		Dominican Republic		Feliz et al. IDIAF unpublished
	16SrIV-B	Mexico	Coconut lethal decline	Harrison et al. (2002b)
		Honduras	Coyol palm decline Coconut decline	Roca et al. (2006)
	16SrIV-D	Mexico	Carludovica palmae, lethal decline Coconut leaf yellowing *Sabal mexicana* foliar decay *Pseudophoenix sargentii* decline	Córdova et al. (2000) Harrison et al. (2002b) Vázquez-Euán et al. (2011) Vázquez-Euán et al. (2011)
		USA	Phoenix palm decline (Texas) Pigmy date palm decline (Florida) Phoenix palm decline (Louisiana)	Harrison et al. (2002c) Jeyaprakash et al. (2011) Singh (2014)
	16SrIV-E	Dominican Republic	Coconut lethal decline	Martínez et al. (2008)
	16SrIV-F	USA (Florida)	*Washingtonia robusta* decline	Harrison et al. (2008)
Ca. *Phytoplasma asteris*	16SrI-B	Colombia	Oil palm lethal wilt	Álvarez et al. (2014)
Ca. *Phytoplasma cocostanzaniae*	16SrIV-C	Kenya, Tanzania	Tanzanian coconut lethal decline	Harrison et al. (2002c)
Ca. *Phytoplasma palmicola*	16SrXXII-A	Mozambique, Nigeria	Lethal yellowing disease	Harrison et al. (2014)

[a]There is no report with subgroup identification

(continued)

Table 9.1 (continued)

Species	16SrDNA Group-Subgroup	Country	Species-Disease Name	Author
Ca. *Phytoplasma palmicola*-related strain	16SrXXII-B	Ghana Côte d'Ivoire	Cape St Paul wilt disease Côte d'Ivoire lethal yellowing	Harrison et al. (2014)
Ca. *Phytoplasma cynodontis*	16SrXIV[a]	Malaysia	Coconut yellow decline	Nejat et al. (2009)
Ca. *Phytoplasma malaysianum*	16SrXXXII-B	Malaysia	Coconut yellow decline	Nejat et al. (2012)
	16SrXXXII-C	Malaysia	Malayan oil palm disease	Nejat et al. (2012)
	16SrXI-B	India	Areca palm yellow leaf disease	Ramaswamy et al. (2013)
Ca. *Phytoplasma oryzae*	16SrXI-B	India	Kerala root wilt disease	Manimekalai et al. (2014)
	16SrXI[a]	India	Oil palm spear rot disease	Sumi et al. (2014)
Ca. *Ptytoplasma cynodontis*	16SrXIV[a]	India	Kerala root wilt disease Areca palm yellow leaf disease	Sumi et al. (2014)
Ca. *Phytoplasma asteris*	16SrI-B	India	Oil palm stunting disease	Mehdi et al. (2012)
Not identified	Not identified	Indonesia	*C. nucifera* Kalimantan wilt	Warokka et al. (2006)
Ca. *Phytoplasma oryzae*	16SrXI[a]	Sri Lanka	Weligama coconut leaf wilt disease (WCLWD)	Perera et al. (2012)
Tentative classification	16SrIV[a]	Papua New Guinea	Bogia coconut syndrome (BCS)	Kelly et al. (2011)
Ca. *Phytoplasma cynodontis*	16SrXIV[a]	Sudan	Date palm disease	Cronje et al. (2000)
Not identified	Not identified	Egypt	Date palm disease	Ammar et al. (2005)
Not identified	Not identified	Egypt	Date palm disease	Al Khazindar (2014)
Ca. *Phytoplasma palmae*	16SrIV-A	Kuwait	Date palm disease	Al-Awadhi et al. (2002)
Ca. *Phytoplasma fraxini*	16SrVI-A	Iran	Date palm disease	Zamharir and Eslahi (2019)
Ca. *Phytoplasma trifolii*	16SrVII-A	Iran	Date palm disease	Zamharir and Eslahi (2019)
Ca. *Phytoplasma australasia*	16SrII[a]	Saudi Arabia	Date palm disease	Omar et al. (2018)
Ca. *Phytoplasma asteris*	16SrI[a]	Saudi Arabia	Date palm disease	Alhudaib et al. (2007)

[a]Unclassified subgroup

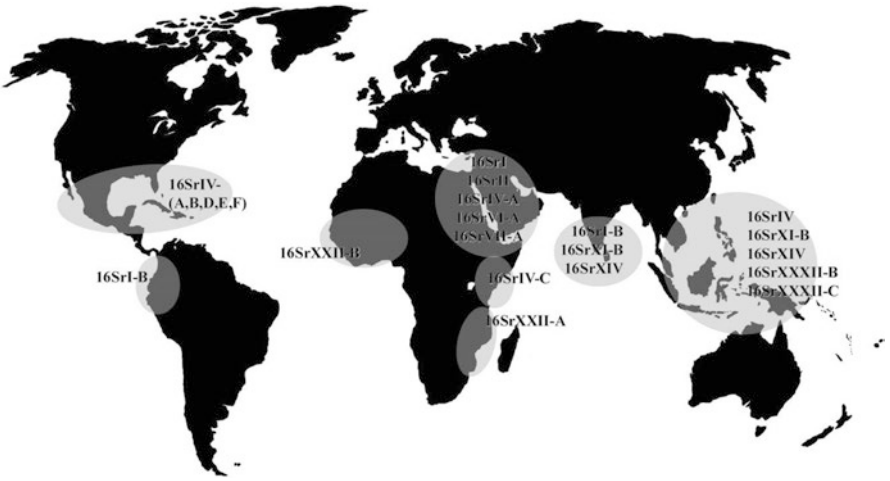

Fig. 9.1 Geographic distribution of phytoplasmas causing lethal yellowing and related diseases in the world. (This map is based on a map reported by Konan et al., COCOTECH Conference, Bali, September 2016)

most apparent when the inflorescence emerges from the spathe. The necrosis increases as the disease progresses, with younger inflorescences showing more extensive necrosis. Most of the male flowers die and no fruit set on affected inflorescences. Yellowing of the leaves usually starts after necrosis has developed in more than two inflorescences. Leaf discoloration due to LY is more rapid than normal leaf senescence. The first leaves to turn yellow are the oldest (lower) ones (Fig. 9.2d). The yellowing advances upward, affecting the younger middle leaves (Fig. 9.2e), and finally the upper ones (Fig. 9.2f). According to McCoy et al. (1983), yellow leaves are turgid and not flaccid as in the case of wilt diseases. Yellow leaves turn brown, desiccate and die. Symptom development was standardized by McCoy et al. (1983) and classified as ten different categories, from zero for healthy palms to nine for dead palms (Table 9.2). This classification system has been proven to be very useful; however, sometimes the pattern varies. For instance, inflorescence necrosis can become noticeable only after leaf yellowing has appeared as observed in Mexico (CICY, Mexico, unpublished) and Guatemala (Mejía et al. 2004).

Once foliar yellowing has reached an advanced stage, a putrid basal soft rot of the youngest leaf (spear) occurs. The spear leaf collapses, followed by an associated rot of the underlying apical meristem, invariably leaving a bare trunk standing (Fig. 9.2g). Roots also show necrosis, which becomes more extensive as the disease progresses (Islas-Flores et al. 1999). The death of the infected palm occurs within 3–6 months after the onset of visible symptoms (McCoy et al. 1983). Symptoms of LY in other palms are generally similar with some variations (McCoy et al. 1983), for example, the Manila palm (*Adonidia merrilllii* Becc.) could present necrosis of the mature leaves or necrosis of the spear leaf or spear leaf opening. However, these symptoms are apparently associated with different subgroups of LY-phytoplasmas

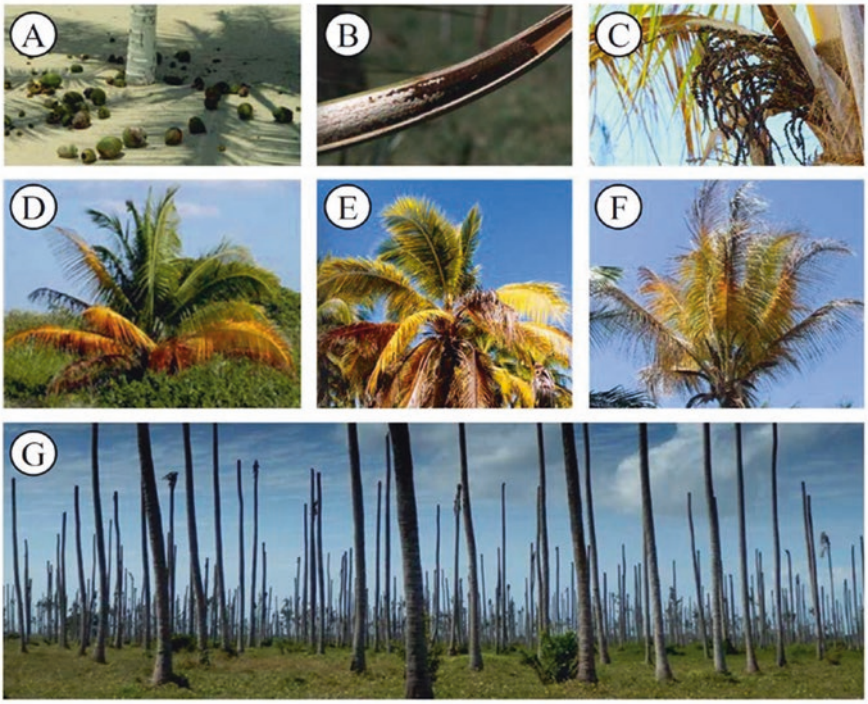

Fig. 9.2 Symptoms of lethal yellowing in fruit-bearing coconut palms: it starts with the premature drop of most of the fruits regardless of their developmental stage (**a**). Then there is blackening of new inflorescences (**b** and **c**). This is followed by yellowing starting with the oldest leaves (**d**), and then advances upward affecting the middle leaves (**e**) and the upper youngest leaves (**f**). Finally, the loss of the crown leaves a bare trunk standing (**g**)

Table 9.2 Rating of lethal yellowing development in coconut (as shown in McCoy et al. 1983)

Category	Stage	Symptoms
Symptomless	0	Healthy or incubating
	1	Nut fall only
Primary[a]	2	One necrotic inflorescence
	3	Two or more necrotic inflorescences
	4	Yellowing in lower leaves only
Yellowing	5	Yellowing in lower and middle leaves
	6	All leaves yellowed, spear leaf good
	7	Spear leaf dead, some green leaves left
Dying	8	Spear leaf dead, all leaves yellowed
	9	Palm dead (telephone pole)

[a]May or may not have a yellow flag leaf in the centre of crown

(Córdova-Lara et al. 2017). Symptoms of coconuts affected by LYDs in West Africa and Tanzania are similar (Yankey et al. 2018) to those described in this chapter for LY in the Americas.

9.4 Causal Agent Identification and Classification

Lethal yellowing in coconut was the first phytoplasma-associated disease found in palm species. Previously known as mycoplasma-like organisms, phytoplasmas were discovered, by electron microscopy, within the phloem vessels of diseased palms but not in healthy palms (Plavsic-Banjac et al. 1972). A cause-and-effect relationship was established when remission of symptoms was obtained in diseased palms when treated with tetracycline. However, it becomes ineffective when applying penicillin antibiotics (McCoy 1982).

As the LY-causing agent is unable to be cultured, its characterization was impossible until the advent of deoxyribonucleic acid (DNA) sequencing and relevant analytical techniques, using in silico Restriction Fragment Length Polymorphism (RFLP) analysis with the online system iPhyClassifier and data from the GenBank sequence database (Zhao et al. 2009). The LY agent has been identified and classified within the 16SrIV group, as well as other strains that are closely related to but distinguishable from the LY agent. Group 16SrIV includes the following subgroup strains: 16SrIV-A, Coconut Lethal Yellowing (LY, Florida USA) (Harrison et al. 2002a); 16SrIV-B, Yucatan Coconut Lethal Decline (LDY, México) (Harrison et al. 2002b); 16SrIV-D, *Carludovica palmata* Leaf Yellowing (CPY, Mexico) (Córdova et al. 2000) and Texas Phoenix Decline (TPD, USA) (Harrison et al. 2002c); 16SrIV-E, Coconut Lethal Decline (CLD, Dominican Republic) (Martínez et al. 2008); and 16SrIV-F, Florida *Washingtonia robusta* Lethal Disease (LD, USA) (Harrison et al. 2008). All are present in the Americas but nowhere else in the world so far, but there is another subgroup that is present in Africa but not in the Americas: 16SrIV-C, Tanzanian Coconut Lethal Decline (LDT, Tanzania) (Harrison et al. 2002c). Tables 9.1 and 9.3 presents more information on each subgroup with other cases within one country or being present in different countries in the Americas. In the case of LYDs outside the Americas, they have also been associated with phytoplasmas of other 16S rDNA groups as shown in Tables 9.1 and 9.3.

9.5 Transmission

According to the surveys conducted in LY-affected areas in Jamaica (Schuiling 1976) and in Florida (Howard and McCoy 1980), the only common species found in coconut palms in both locations was a leafhopper (*Haplaxius crudus* Van Duzee, 1907). This pest belongs to Auchenorrhyncha, sub-order of Homoptera, and is the most phytoplasma-associated vector. In addition, the apparent rate of spread of LY

Table 9.3 Palm species (other than coconut) and non-palm species hosting phytoplasmas associated with LY and related diseases

Plant species	Common name	Country	Strain	Author
Acrocomia aculeata	Coyol palm	Honduras	16SrIV-B 16SrIV-D	Roca et al. (2006) Ntushelo et al. (2013)
Adonidia merrillii	Manila palm	USA	16SrIV-A	Harrison and Oropeza (2008)
		Mexico	16SrIV-A 16SrIV-D	Córdova-Lara et al. (2017)
Aiphanes lindeniana	Ruffle palm	USA	16SrIV	Harrison and Oropeza (2008)
Alagoptera arenaria	Seashore palm			
Arenga engleri	Dwarf sugar palm			
Borassus flabellifer	Palmyra palm			
Caryota mitis	Giant fishtail palm	Puerto Rico	16SrIV-D	Rodriguez et al. (2010)
Caryota rumphiana	Round leaf palm	USA	16SrIV	Harrison and Oropeza (2008)
Caryota urens	Wine palm			
Chelyocarpus chuco	Round leaf palm			
Cocos nucifera	Coconut	–	–	See Table 9. 1
Coccotrinax readii	Nakás palm	Mexico	16SrIV-A	Narvaez et al. (2006)
Corypha taliera	Buri palm	USA	16SrIV	Harrison and Oropeza (2008)
Crysophyla warsecewiczii	Guáguara palm			
Cyphophoenix nucele	Lifou palm			
Dictyosperma album	Princess palm			
Dypsis cabadae	Cabada palm			
Dypsis decaryi	Triangle palm			
Elaeis guineensis	Oil palm	Colombia	16SrI-B;	Álvarez et al. (2014)
		Mozambique	16SrXXII-A	Bila et al. (2015)

Gaussia attenuata	Puerto Rican Gaussia palm	USA	16SrIV	Harrison and Oropeza (2008)
Howea belmoreana	Belmore sentry palm			
Howea forsteriana	Kentia palm			
Hyophorbe 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	USA	16SrIV-A 16SrIV-D	Harrison et al. (2002, 2008)
			16SrIV-D	Ntushello et al. (2013)
Phoenix dactylifera	Edible date palm	USA	16SrIV-A 16SrIV-E 16SrIV-F	Harrison et al. (2008)
Phoenix reclinata	Senegal date palm	USA	16SrIV-D	Harrison et al. (2008, 2009)
Phoenix rupícola	Cliff date palm			
Phoenix silvestris	Silver date palm			
Phoenix roebelenii	Pygmy date palm			
Pritchardia pacifica	Fiji island fan palm	Mexico	16SrIV-D	Narvaez et al. (2017)
Pritchardia affinis	Kona palm	USA	16SrIV	Harrison and Oropeza (2008)
Pritchardia pacifica	Fiji island fan palm			
Pritchardia remota	Remota loulu palm			
Pritchardia thurstonii	Thurston palm			

(continued)

Table 9.3 (continued)

Plant species	Common name	Country	Strain	Author
Pseudophoenix sargentii	Florida cherry palm	USA	16SrIV-D	Harrison et al. (2008)
		Mexico		Vázquez-Euán et al. (2011)
Ravenea hildebrantii	Hildebrandt's palm	USA	16SrIV	Harrison and Oropeza (2008)
Roystonea regia	Cuban royal palm	Puerto Rico	16SrIV-D	Rodriguez et al. (2010)
		Mexico		Narvaez et al. (2016)
Syagrus schizophylla	Arikury palm	USA	16SrIV	Harrison and Oropeza (2008)
Syagrus romanzoffiana	Queen palm	USA	16SrIV-D	Harrison et al. (2008) and Ntushelo et al. (2013)
Sabal palmeto	Cabbage-palm	USA	16SrIV-D	Harrison et al. (2009)
Sabal mexicana	Mexican palmetto	Mexico	16SrIV-A 16SrIV-D	Vázquez-Euán et al. (2011)
Trachycarpus fortunei	Windmill palm	USA	16SrIV	Harrison and Oropeza (2008)
Thrinax radiata	Florida thatch palm	México	16SrIV-D	Narváez et al. (2006)
Veitchia arecina	Montgomery's Palm	USA	16SrIV	Harrison and Oropeza (2008)
Washingtonia robusta	Mexican fan palm	USA	16SrIV-D 16SrIV-F	Harrison et al. (2008) and Ntushelo et al. (2013)
Non-palm species				
Pandanus utilis	Common screwpine	USA	16SrIV	Thomas and Donselman (1979)
Carludovica palmata	Panama hat plant	Mexico	16SrIV-D	Córdova et al. (2000)
Emilia fosbergii	Florida tasselflower	Jamaica	16SrIV-A	Brown et al. (2008)
Synedrella nodiflora	Nodeweed			
Stachytarpheta jamaicensis	Blue porterweed	Jamaica	16SrIV-E (tentative)	Brown and McLaughlin (2011)
Macroptilium lathyroides	–			
Cleome rutidosperma	Fringed spider flower			
Paspalum vaginatum	Seashore paspalum	Costa de Marfil	16SrXXII-B	Arocha-Rosete et al. (2016) and Yankey et al. (2018)
Pennisetum pedicellatum	Desho grass			
Stachytarpheta indica	–			
Scoparia dulcis	Licorice weed			
Phyllanthus muellerianus	–			
Diplacrum capitatum	–			
Manihot esculenta	Cassava	Costa de Marfil	16SrXXII-B	Kra et al. (2017)

was decreased in areas where *H. crudus* populations were reduced by insecticide treatment (Howard and McCoy 1980). It was also noted that the populations of *H. crudus* in heavily affected areas were 40 times higher than in LY-free areas of Florida (Howard 1980). When coconut and other palm species, within insect-proof cages, were exposed to *H. crudus* adults captured from palms in LY-affected areas, transmission of LY to most test palms occurred within 34 months. In contrast, similar palms which were not exposed to *H. crudus* remained healthy (Howard et al. 1983). Furthermore, a Polymerase Chain Reaction (PCR)-based detection of LY-phytoplasma infection was reported in native *H. crudus* in Florida. Taken together, these studies have indicated the significance of this planthopper as a vector for LY in Florida.

Studies have also been conducted in the identification of vectors of LY phytoplasmas in other countries. In Jamaica, positive LY-phytoplasma detection has been confirmed in *H. crudus* and in another homopteran: *Cedusa* sp., captured in LY-affected sites (Brown et al. 2006). In the Yucatan peninsula of Mexico, where LY has been a devastating factor for cultivation of coconut and other palm species, wild *H. crudus* insects were captured from palm foliage and showed positive detection for 16SrIV phytoplasmas at a proportion of 2.7% (of 2726 insects analysed). Also, the detection was positive in both male and female insects, with a higher proportion found in males (Narvaez et al. 2018). In silico, RFLP and phylogenetic analyses of PCR-amplified Ribosomal DNA (rDNA) products showed that *H. crudus* insects could individually harbour one of three strains: 16SrIV-A, 16SrIV-D or 16SrIV-E, a strain diversity that has also been found in palms affected by LY-type disease syndromes in this part of Mexico (Narvaez et al. 2018). When these *H. crudus* wild insects were tested as vectors of LY phytoplasmas in insect-proof cages containing LY susceptible *Pritchardia pacifica* palms, positive transmission occurred, *P. pacifica* palms developed a LY-type syndrome and died. The phytoplasma associated strain was found as 16SrIV-A in some palms, while it was shown as 16SrIV-D in other palms (CICY, Mexico, unpublished). Similar results were obtained when using an in vitro system for testing vector transmission. After an introduction of *H. crudus* for 1 month, micropropagated coconut plantlets developed leaf yellowing. The PCR analysis of both plantlets and insects confirmed positive results and the phytoplasma strain found was the same in both plantlets and insects (CICY, Mexico, unpublished). In Tabasco, Mexico, insects of *H. crudus* captured from LY-affected palms were found to be positive with PCR detection for 16SrIV phytoplasmas. Other homopteran species, *Haplaxius skarphion*, *Haplaxius cadwuellii*, *Oeclus snowii* and *Persis foveastis*, also tested positive for 16SrIV phytoplasmas (CP, Mexico, unpublished). The results obtained in the USA, Mexico and Jamaica confirmed that *H. crudus* is a vector of 16SrIV phytoplasmas. However, there might also be other vectors involved in the transmission of these phytoplasmas.

With regard to environmental conditions favouring vector populations, a study was conducted on the wild populations of *H. crudus* growing on St Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze), Bahia grass (*Paspalum notatum* Flüggé) and Bermuda grass (*Cynodon dactylon* (L.) Pers.). Noticeably, significantly

higher numbers of adults and nymphs were collected on St Augustine grass (Reinert 1980). Another study by Howard (1990) also showed a preference of *H. crudus* for St Augustine grass over other grasses. In a coconut pathosystem in southern Mexico, para grass (*Brachiaria mutica* (Forssk.) Stapf), finger grass (*Eutachys petraea* (Sw.) Desv.), signal grass (*B. humidicola* (Forssk.) Stapf) and panic grass (*Panicum laxum* L.) were identified as the principal host species for *H. crudus* nymphs (Ramos-Hernández et al. 2018). It has been shown that grasses and St Augustine grass favour *H. crudus* populations. Therefore, management of the grasses under palm plantings is a potential method for suppressing *H. crudus* populations and thus reducing the spread of LY (Howard 1990).

The investigation of putative vectors of LYDs in Africa has led to the PCR-based detection of phytoplasmas in different insect species. In Tanzania, positive detection was obtained in the planthoppers: *Diostrombus mkurangai* and *Meenoplus* sp. (Mpunami et al. 2000), but transmission studies have not been successful. In Ghana, the presence of Cape Saint Paul Wilt Disease (CSPWD phytoplasma and transmission studies (when caged with coconut palms) were carried out with insects of four *Diostrombus* spp. and *Myndus adiopodoumeensis.* Negative results were confirmed in all PCR tests for *M. adiopodoumeensis* and exposed coconut palms. In the case of the *Diostrombus* spp., one coconut plant and one *D. mayumbensis* insect were positive to CSPWD phytoplasma. However, the coconut plant did not develop symptoms and later PCR testing was negative (Philippe et al. 2009). Although the results from this study did not support a definitive role of *D. mayumbensis* as a CSPWD phytoplasma vector, the insect should still be considered as a potential vector. In fact, further research in LY transmission needs to be undertaken with this species.

In Côte d'Ivoire, insects of *Nedotepa curta* Dmitriev (Cicadellidae: Typhlocybinae: Erythroneurini) were found to be positive not only for 16SrXXII-B phytoplasma (causing Côte d'Ivoire lethal yellowing, CILY) but also to 16SrI phytoplasma. In fact, both phytoplasmas were found as mixed infections in a group of coconut palms (Kwadjo et al. 2018). In Mozambique, early PCR screening showed positive detection of coconut lethal yellowing disease (CLYD) phytoplasmas in *Platacantha lutea* revealing this derbid as a potential insect vector of LYD phytoplasma in northern Mozambique (Dollet et al. 2011). Further research in Mozambique showed positive detection of CLYD phytoplasmas in *Diostrombus mkurangai* and that this insect could also be a potential vector of CLYD (Bila et al. 2017). These findings suggest potential vectors of LYDs in these African countries, but their capacity for transmission is yet to be confirmed.

In the case of Asian countries, research has been conducted to identify vectors of LYDs. The phytoplasmas (then known as Mycoplasma-like organisms, MLOs) causing Kerala wilt disease in India were found to be transmitted in cages by insects of *Stephanitis typica* to coconut plants. The detection was undertaken with 4′,6-diamidino-2-phenylindole (DAPI) staining, electron microscopy and serodiagnosis (Mathen et al. 1990). More recent studies reported that positive cage transmission of phytoplasmas can cause root wilt disease (16SrIX) through insects of *Proutista moesta* (Rajan 2011). These results suggest the vector role of *S. typica* and

P. moesta. In Sri Lanka, investigation of vectors associated with Weligma Coconut Leaf Wilt Disease (WCLWD) showed that eight homopteran species and a hemipteran species were positive to PCR-based detection. The DNA sequence was like WCLWD phytoplasma sequence, suggesting them as putative vector species of WCLWD (Kumara et al. 2015). In the case of Bogia Coconut Disease (BCD) in Papua New Guinea (PNG), positive Loop Mediated Isothermal Amplification (LAMP) was obtained in feeding solution and head tissue of insects from the families: Derbidae, Lophopidae, Flatidae and Ricaniidae and nested-PCR sequences obtained were identical to *Cocos nucifera* BCD phytoplasma sequences from GenBank (Lu et al. 2016). For these cases in Sri Lanka and PNG, further research is required to confirm the capacity of these insects for transmission of phytoplasmas.

9.6 Spread

Studies on gradients of LY spread in outbreaks of the disease were carried out in coconut groves in Yucatan where the disease was spreading from east to west. The results showed that as the proportion of infected palms in an outbreak increased, the distance of the disease spread from the outbreak increased and did so as an east-west symmetrical gradient (Góngora-Canul et al. 2004). Also, in Yucatan, coconut plants within a coconut grove affected by LY were studied to define the pattern of spread by following appearance of visual symptoms. The results showed that spread was randomly distributed (first 10 months) and afterward aggregates started forming, until the distribution of symptomatic palms was uniform in the whole grove (Pérez-Hernández et al. 2004). In contrast, if infected palms were searched using PCR detection, aggregate formation was already occurring at a time when symptom development was showing a random distribution (Góngora-Canul et al. 2004). In addition, studies carried out using analysis of spatial autocorrelation following visual symptom development in a coconut grove, where palms were separated 8 m from each other, showed that an infected palm could infect palms that are close to it as far as 64 m (Góngora-Canul et al. 2004).

There is also another type of spread that has been referred to as 'jump-spread' because it is associated with longer distances of up to several tens of kilometres, where a new outbreak starts with no diseased plants in between. The fact that this is occurring, supports the participation of a flying insect vector or vectors and this may be affected by the wind. Studies on long-distance spread of LY that were carried out in Yucatan showed that the gradients of LY east-west spread were asymmetrical, larger to the west than the east, coinciding with the wind direction (Pérez-Hernández et al. 2004). When patterns of spread of CSPWD were studied in coconut plantations in Ghana, it first occurred randomly in isolated palms, spreading through the entire plot in patches and then steadily to the entire plantation (Dery and Philippe 1997). And there were also jump-spreads of varying distances (Dery and Philippe 1997).

It is likely that there may be alternative paths of pathogen spread associated with human activities, particularly for long-distance dispersal that involves hundreds of kilometres. When LY first appeared in the Cancun area in Mexico and in the island of Roatan in Honduras, the disease was in both cases hundreds of kilometres away. It is suspected that LY arrived at these two sites because ornamental plants or plant parts were transported from an LY-affected region to these two tourist resorts while they were being developed; of course, this remains to be confirmed. Similarly, a particular risk could arise by taking coconut fruit from an LY-affected region or country, to an LY-free region or country. Phytoplasma DNA can be detected by PCR in embryos of fruits from LY-diseased coconut palms. In sectioned tissues from positively testing embryos, the distribution of phytoplasma DNA was shown by in situ PCR to be localized to areas corresponding to the plumule and cells enclosing it (Córdova et al. 2003). The presence of phytoplasma DNA in embryos of fruits at different stages of development from LY-diseased coconut palms was subsequently demonstrated (Oropeza et al. 2011). Research also showed that plantlets obtained by in vitro germination of embryos from seed of LY-diseased palms were infected with LY phytoplasmas as determined by PCR detection, whereas plantlets obtained from seed from LY-free palms were disease free (Oropeza et al. 2017). Therefore, an alternative path of spread is likely if seed from LY-affected areas is taken and germinated in LY-free areas.

9.7 Plant Host Species

A great number of plant species can be infected with phytoplasmas associated with LY or related diseases. Table 9.3 shows 49 palm species (besides coconut) and 14 non-palm species that have been reported as affected by phytoplasmas with group and subgroup having been identified for most of them. Most of the susceptible palm species are used as ornamentals, but some hold economic value such as oil, date and coconut palm.

A few palm species have been reported that could be infected separately by two different phytoplasmas of the same group: *A. aculeata* with 16SrIV-B or 16SrIV-D (Ntushelo et al. 2013; Roca et al. 2006), *A. merrillii* with 16SrIV-A or 16SrIV-D (Córdova-Lara et al. 2017), *S. Mexicana* with 16SrIV-A or 16SrIV-D (Vázquez-Euán et al. 2011), *P. canariensis* with 16SrIV-A or 16SrIV-D (Harrison et al. 2002, 2008, Ntushello et al. 2013) and *W. robusta* (16SrIV-D or 16SrIV-F; Harrison et al. 2008; Ntushelo et al. 2013). Also, there is a case of a species infected with phytoplasma belonging to different groups, *E. guineensis* with 16SrI-B (Álvarez et al. 2014) or 16SrXXII-A (Bila et al. 2015), each occurring in different countries. We also have the particular case of coconut with separate infections reported with as many as five phytoplasma subgroups: 16SrIV-A, 16SrIV-B, 16SrIV-C, 16SrIV-D and 16SrIV-E (Harrison et al. 1999; Harrison and Oropeza 2008; Martínez et al. 2008; Myrie et al. 2014; Ntushelo et al. 2013; Roca et al. 2006); however, subgroup 16SrIV-A is the predominant one and most damaging (Harrison and Oropeza 2008).

Fourteen non-palm species have been reported with the presence of LY-phytoplasma. Seven species can be infected with the group 16SrIV: *P. utilis* (Thomas and Donselman 1979), *E. fosbergii* and *S. nodiflora* (16SrIV-A; Brown et al. 2008), *C. palmata* (16SrIV-D; Córdova et al. 2000), *S. jamaicensis, M. lathyroides and C. rutidosperma* (16SrIV-E; Brown and McLaughlin 2011). In addition, seven species with the group 16SrXXII: *P. vaginatum*, *P. pedicellatum*, *S. indica*, *S. dulcis*, *P. muellerianus*, *D. capitatum* and *M. esculenta* (all with the subgroup 16SrXXII-B).

It is important to keep in mind that there are several reports producing lists of palm species of which individuals have been found susceptible to LY (Harrison and Oropeza 2008). However, most of these species have not been tested or observed for their level of susceptibility or resistance as a population.

Although there are some cases such as *E. guineensis* in Colombia (Álvarez et al. 2014), *P. dactylifera* in USA (Harrison et al. 2002c) and *P. pacifica* in Mexico (Narvaez et al. 2017), they have been shown to be very susceptible as a population, since most of the individuals were destroyed due to LY or LYDs. There are some species that are known not to be susceptible as only a few individuals die (probably less than 5%) without affecting the population size. In fact, such species, e.g. *S. Mexicana*, *Thrinax radiata* and *Coccothrinax readii*, could be acting as reservoirs of phytoplasmas (Narvaez et al. 2006; Vázquez-Euán et al. 2011).

9.8 Sampling of Tissue and Insects

9.8.1 Tissue Sampling

Regardless of analytical technique, detection of phytoplasmas in infected coconut plants, or other plant species, was typically carried out on tissue samples from very young leaves, unopen inflorescences or apical meristems. However, these tissues are very difficult to obtain in fully mature plants. With the advent of the very sensitive molecular methods for phytoplasma detection, the more easily obtained tissues from the lower part of the trunk (using a drill) were tested as a source of phytoplasma DNA and the results were positive (Córdova 2000). The comparative advantage of trunk shavings was confirmed by Oropeza et al. (2011). They studied the distribution of LY phytoplasmas in different parts (trunk, young leaves, inflorescences, stem apex and root apex) of infected coconut plants during symptom development, starting with plants not showing symptoms yet (stage 0, according to McCoy et al. 1983) to the stage in which they have advanced yellowing of the leaves (stage 6, according to McCoy et al. 1983). The results showed that detection by nested-PCR could be obtained in all plant parts studied, even in symptomless infected plants. However, frequency of detection was low in all parts except in trunk where it was high already at stage 0. Furthermore, the frequency of detection in all parts decreased as symptomatology progressed, except in the trunk. Detection in the

trunk is still possible even when plants have lost the crown (CICY, unpublished). Therefore, trunk has become the source of choice for tissue sampling for phytoplasma detection for coconut and other plant species.

Trunk sampling is easy. One protocol according to Oropeza et al. (2010) involves the following steps: Drill a hole in the trunk, 10 cm deep with a drill bit (usually 5/8 inch in diameter) and approximately 1.5 m above the ground (Fig. 9.3a). The shavings are collected in a plastic bag avoiding the contact of these with the hands (Fig. 9.3b and c). Once the sample has been taken, the drill should be washed with 3% sodium hypochlorite (Fig. 9.3d) and then rinsed with sterile water. When finished, the hole should be blocked with a wood plug (Fig. 9.3e and f), it is advisable to apply insecticide over the sealed hole for additional protection against pests and pathogens.

The samples contain wood shavings as well as sap content where phytoplasma DNA is present in larger amount in comparison to other parts of the palm (Córdova et al. 2014). These trunk samples are adequate for standard laboratory PCR or LAMP analysis, and also for in-field detection using portable thermocycler machines coupled with dipstick technology (Zou et al. 2017).

9.8.2 Insect Capture

For the collection of insects, plants having easy-to-reach foliage should be chosen. In the case of *H. crudus*, it is easier to find more insects in the morning, no later than 10 a.m. However, this could depend on the insect species and local conditions. Insects are captured from the lower side or abaxial side of the leaves of palms (Fig. 9.3g). If the purpose is capturing insects that are infected with 16SrIV phytoplasmas, it is more convenient to collect insects from palms that are showing symptoms and have already been determined as positive with PCR detection. If the purpose is collecting phytoplasma-free insects, collect them from plants that are in an area that is free of LY. It is very important that the insects are captured using a system designed for trapping them. The system may include a bottomless tube inside a larger tube, in order to capture without causing any damage (Fig. 9.3h). Once captured, they can be kept in tubes within a cool container. Identification at the laboratory can be carried out under a stereoscope using key morphological characteristics (Kramer 1979; Triplehorn and Johnson 2005). Some of the insects should be sent to experts for confirmation of identity.

9.9 Detection and Diagnosis of LY Phytoplasmas

Symptoms are the first evidence for diagnosis of LY phytoplasmas. Abnormal falling of fruit, necrosis of inflorescences and yellowing of mature leaves could be indicative of the presence of LY-related phytoplasmas in palms. However, the

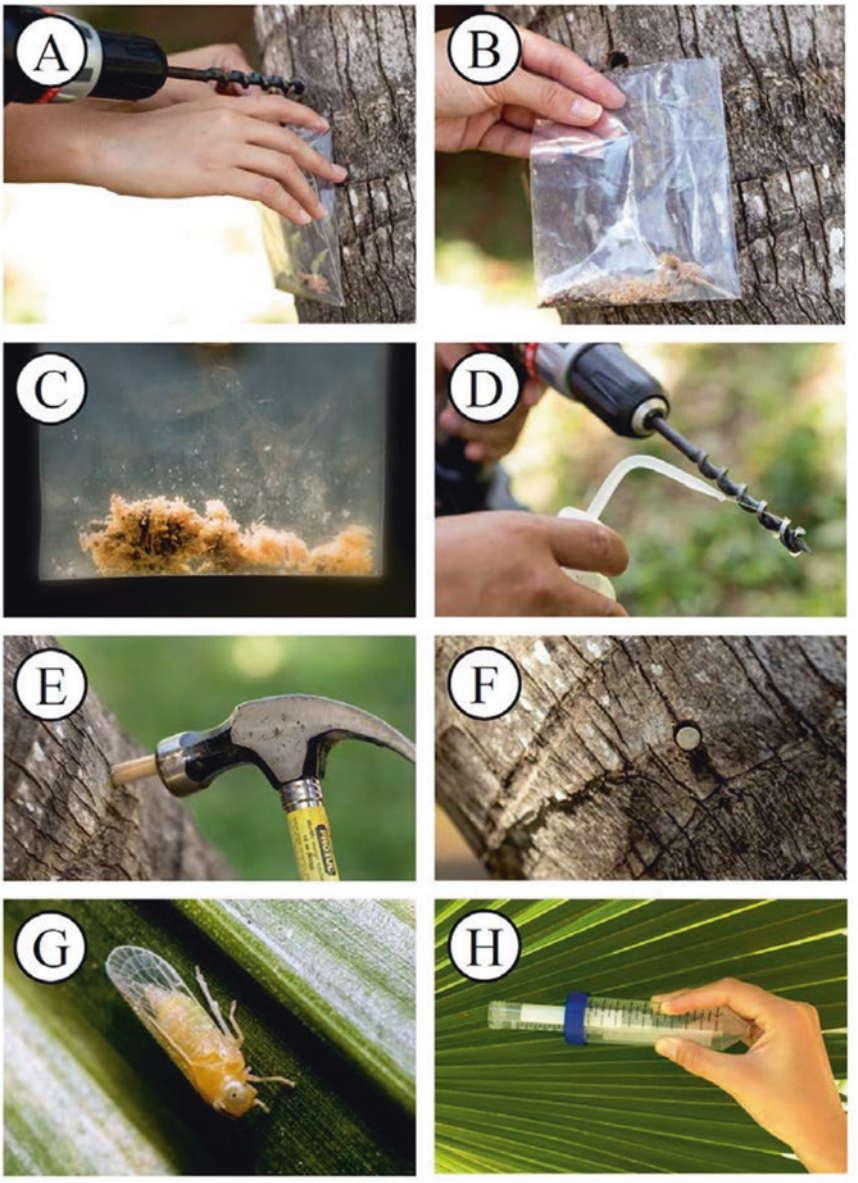

Fig. 9.3 Trunk sampling for phytoplasma detection: Drill a hole in the trunk at approximately 1.5 m above the ground (**a**). Collect the shavings in a plastic bag (**b**, **c**). Wash the drill with sodium hypochlorite (NaClO) at 3% (**d**). Rinse with sterile water. Block the hole with a wood plug (**e**, **f**). Insect capture: Find an insect of interest (*Haplaxius crudus* shown) on the abaxial side of a palm leave (**g**) and then capture it using a tool as the one shown (a bottomless tube inside a larger tube) with no aspiration involved to avoid damaging the insect (**h**)

disease can only be confirmed through specific diagnostic tools. In the past, transmission electron microscopy was used to confirm the presence of LY phytoplasmas (Plavsic-Banjac et al. 1972). It helped visualize them as ovoid or filamentous cells, living in the phloem of palms. However, this technique was very laborious and time-consuming and only few samples could be analysed at once. Despite these disadvantages, this technique was utilized from the 1970s to the early 1990s (Howard, 1995). The advent of molecular techniques in recent years provides better solutions for diagnosis.

The first tests of molecular diagnosis were using DNA probes marked with radioactivity (^{32}P) or fluorescent molecules that hybridize with the LY DNA. This technique allowed the detection of a great number of samples using the dot blot analysis. However, the sensitivity and specificity were limited due to low titres of phytoplasma in the samples (Harrison et al. 1994). These problems were solved when the PCR technique was applied for phytoplasma detection. It started with the development of primers that amplify rDNA from universal phytoplasma sequences and later with the development of specific primers for LY phytoplasma (Harrison et al. 2002b). This technique allowed detection of multiple samples with specificity and rapidity; however, due to the low titre of phytoplasma it was necessary to use two rounds of DNA amplification (nested-PCR) using universal and specific primers.

The establishment of quantitative PCR (qPCR) and TaqMan technology (hydrolysis probes) that enable the detection of DNA amplification in real-time using a specific probe labelled with a fluorophore helped to increase the sensitivity and specificity of phytoplasma detection, using only one round of DNA amplification. For detecting LY and LYD phytoplasmas, TaqMan probes were developed based on their 23S ribosomal gene using different sets of specific primers (Hodgetts et al. 2009). For LY phytoplasma that affect palms in the Americas, a specific qPCR assay was developed by Córdova et al. (2014), using a TaqMan probe and primers based on the 16S ribosomal gene. This assay enabled the absolute quantification of phytoplasma, showing that trunk, root meristem and immature inflorescences had the highest level of phytoplasma in coconut palm. This technique has been improved using two TaqMan probes targeting different genes (16S rRNA and GroEL) and labelled with different fluorophores that allow detection and discrimination of two strains of LY phytoplasma (16SrIV-A and -D) at the same time (Córdova et al. 2019). Other authors have developed a similar technique using SYBR Green (Asymmetrical Cyanine Dye) Fluorophore that binds to the double helix of amplified DNA using the melting point analysis of this molecule to discriminate the LY strains (Bahder et al. 2017). More recently, a new technique derived from real-time PCR has been developed and is called digital PCR. The advantage of this technique is that the detection and quantification is done by microdroplets. This provides a higher sensitivity and accuracy than the real-time PCR. Bahder et al. (2018) have developed a digital PCR protocol for LY phytoplasma detection that is more sensitive than the real-time protocol reported by Córdova et al. (2014). This technique opens up new research avenues because it would help detect very low titres of phytoplasma in plants and insects, providing a new tool for tackling this disease. An

additional technique LAMP has been progressively used to detect plant pathogens in recent years. This technique consists in the amplification of six different targets of DNA that are amplified at isothermal conditions (60–65 °C) in a short time (usually 1 h or less). The addition of loop primers increases the rapidity and sensitivity of the technique. It has the potential to be simple and rapid and also requires minimal equipment and could be used in field conditions. A protocol using this technique has been reported for detection of phytoplasma of group 16SrXXII that affect palms in Africa (Tomlinson et al. 2010) and phytoplasma of group 16SrIV in insects of different coconut plantations affected with BCS in Papua New Guinea (Lu et al. 2016).

9.10 LY Resistance Screening

During the 1950s, there were great losses of coconuts in Jamaica, prompting field screening for LY resistance of coconut germplasm. Several cultivars were tested and two were selected: the Malayan Yellow Dwarf (MYD) with a very low mortality of 4% and the Panama Tall (PT) with an intermediate mortality of 44%; they were also crossed to produce the MYD × PT hybrid (Maypan), combining the LY resistance of MYD and the better agronomic characteristics of PT. Since then, the Maypan has been extensively planted in Jamaica and other countries. Unfortunately, since the 1990s, the MYD, PT and the Maypan have been dying from LY in proportions greater than expected (Broschat et al. 2002). Genetic contamination was evaluated in PT populations in Jamaica and found to be present (Baudouin et al. 2008), and similar results were obtained for MYD populations (Lebrun et al. 2008). However, it was believed to be insufficient to explain the recent LY outbreaks in these varieties and the Maypan hybrid produced from them. Hence, there are likely to be other causes. The above-mentioned authors believe that these coconut materials cannot be resistant to LY for the current situation in Jamaica.

Similarly, an approach for crossing the MYD with Pacific Tall (PT) ecotypes has been used in other countries, including Mexico where several such hybrids were produced by Instituto Nacional de Investigaciones Forestales y Agrícolas y Pecuarias (INIFAP, México). As a result, these palms have successfully survived LY for decades. The coconut germplasm on the Pacific coast of Mexico was originally introduced from East Asia and the Pacific between sixteenth and nineteenth centuries (Zizumbo-Villarreal 1996). Hence, genetic diversity and probably LY resistance could be expected. Also, coconut populations representing the genetic diversity in Mexico, particularly of the Pacific side, were screened for mortality to LY starting in 1989. The test included the Atlantic Tall (AT) and MYD as references, and 15 PT populations that were characterized and grouped into three ecotypes: MXPT1, MXPT2 and MXPT3 (Zizumbo-Villarreal et al. 2008). The MYD showed the lowest mortality and AT the highest, and MXPT1 and MXPT2 had a mortality similar to that of MYD.

Encouraged with these results, further screening by INIFAP, CP and CICY has been carried out jointly, with introduction of germplasms originally from East Asia and the Pacific. These include the first introduction of six hybrids of which two stand out: Malayan Red Dwarf × Vanuatu Tall (MRD × VTT) and Malayan Red Dwarf × Tagnanan Tall (MRD × TGT); and a second introduction with 12 varieties, currently under evaluation. The MXPT1 and MXPT2 ecotypes have been used as male parents with MYD as a female parent for hybrid production. The ecotypes and the hybrids which were used in replanting programs for nearly 20 years have survived, without being associated with any outbreak of LY so far. It is still the case even when they are cultivated in areas where different LY phytoplasma strains exist (Harrison et al. 2002b). This situation contrasts with what has happened in Jamaica with the supposedly resistant MAYPAN and parents MYD and PT.

Screening for resistance to CSPWD has also been reported in Ghana and the results showed a certain level of susceptibility for most of the varieties evaluated. West African Tall was the most susceptible, given that most of this type died. In contrast, most of the Sri Lankan Green Dwarf (SGD) palms have survived (Dery et al. 2008).

Given the information documented above, affected countries need to continue or start screening for resistance to LY or LYDs in local coconut germplasm and introduced germplasm. Given field screening for LY resistance takes a long time (up to 10 years or more), without the guarantee that within a trial disease incidence will build up to adequate levels, or natural calamities or man-made restrictions could end abruptly with the trial, we also need to develop more rapid and precise ways to assess susceptibility and resistance of coconut germplasm to phytoplasmas associated with LY or LYDs.

9.11 Translating Knowledge into Practical Use

In order to deal with LY (and related diseases), it is useful to consider what we know about the disease and other relevant aspects, particularly in relation to those species that are socially and economically important. For instance, one should look at the potential source of the pathogen, its diversity and geographic distribution, whether it needs vectors, as well as the habits of the vectors (their biology, factors for spread, the plant host species and plant-pathogen-vector interactions) and perhaps most importantly if there is resistant germplasm. Based on the aforementioned information on LY (and LYDs in general), the first action is perhaps to produce a contingency plan of general use. However, that would need to include countries or areas where these diseases are not currently present. Measures have to be taken to stop entry of infected biological materials (plant or animal). There is a contingency plan for LY in Spanish published by Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA) (Oropeza et al. 2010), but it needs updating and refinement; however, it could be a convenient starting point.

There are several phytoplasmas in the Americas; however, the only one that is devastating for coconut so far is 16SrIV-A. Occurrences of coconut palms affected by other subgroup phytoplasmas (B, D and F) have also been observed in very small outbreaks in Mexico and Dominican Republic but they have not spread. It is important to monitor them, and new incursions, to define immediately if it is a phytoplasma disease. This could be done via gathering information on symptoms and context and using an in-field detection technique based on qPCR or LAMP (Córdova et al. 2014; Tomlinson et al. 2010 respectively), alternatively sampling can be carried out by a method such as the use of dipstick (Zou et al. 2017) (see tissue sampling above) and samples sent to a laboratory. Diseased plants then need to be destroyed. If we are dealing with a case in a region or country where LY or an LYD is not yet present, it would be very important to analyse surrounding symptomless palms and try to contain the outbreak (see Oropeza et al. 2010). Every step and the participants needed for these action(s) should to be very well defined as part of a contingency plan.

Importantly, special care should be taken to avoid the introduction of infected plant material or insects from a disease affected region or country to a disease-free region or country. For instance, bringing a nut, perhaps as a souvenir, from a country with LY to a country without. If that nut has an embryo infected and the nut is germinated, this could be a risk of introduction and spread of the disease. This would be worse if the material is from an LYD-affected country in one continent and moved to a country in another continent, where the disease is not present. So, it is very important that strict measures are taken by countries to avoid such occurrences.

In the cases where LY is already present in a country and spreading, measures should be taken to reduce the economic damage that can result. This could be in the form of an integrated disease management package. The Coconut Industry Board in Jamaica has implemented such a package that consists of the following: *(a) Surveillance.* This could be done while carrying out the different actions for managing the plantation, such as harvesting fruit. So, when an early symptom or symptoms are observed in a plant, it should be reported. *(b) Elimination of the symptomatic plant.* This should be done as immediately as possible after the symptomatic plant was spotted. The plant cannot be cut down and left there, since leaves that are still alive will continue being a source of phytoplasmas for vector insects feeding on them. Sampling is an option that could be convenient for later analysis. *(c) Replanting.* This should be done systematically to keep the plantation's plant density. It should be done using germplasm selected for resistance or with some level of resistance to LY. It would not be convenient to use susceptible coconuts such as the AT variety. *(d) Weeding.* Keeping the plantation free of weeds is important because it involves destruction of Gramineae plants that could host vector insects, as well as other plant species that could host the phytoplasmas as observed in Jamaica (Brown and McLaughlin 2011). *(e) Control of vectors.* Use of convenient agents for this purpose is important to reduce as much as possible the population of insects that could be vectors for the phytoplasmas. *(f) Health.* Provide the plants with good maintenance and nutritious conditions, free of other diseases and pests as much as possible. This integrated disease management package is being applied in Jamaica

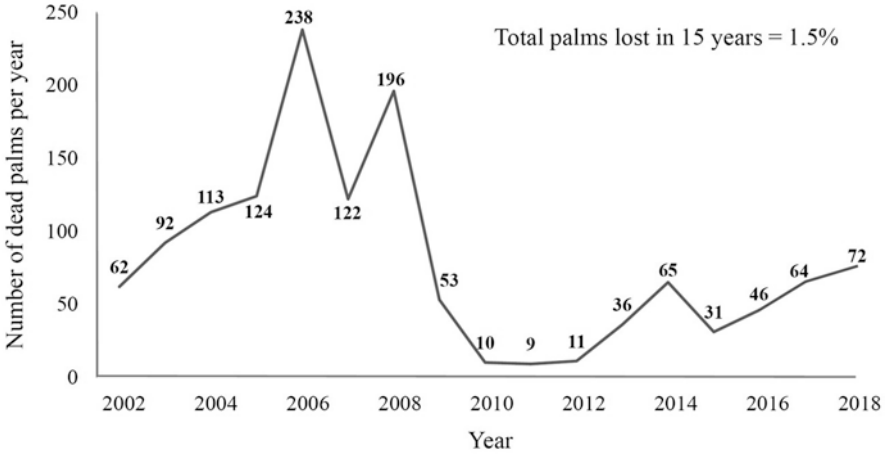

Fig. 9.4 Integrated LY management in Black's Farm, St Thomas, Jamaica, where there are nearly 80,000 plants producing fruits which are used for different products, including bottled water and virgin oil. The loss throughout 15 years does not exceed 1.5% of the 80,000 plants in the farm

on Black's Farm where about 80,000 plants have been cultivated for more than 10 years and losses due to LY have been kept below 0.5% in most years (Fig. 9.4).

However, in other countries, such as Mexico, where LY resistant ecotypes have been found and used for more than 20 years, this resistant germplasm is the main factor for dealing with LY. With the use of resistant germplasm and the addition of integrated disease management packages (described above) there would be a much lesser risk of losses due to LY.

9.12 Future Perspectives and New Venues of Research

Perhaps the most important lesson is that different institutions need to work together to develop a global strategy for identifying the resistance of coconut genotypes to the LY and LYD phytoplasmas that are devastating coconut cultivation. This could be based on studies such as that of Baudouin and Lebrun (2009) that theoretically identified the level of mortality LY could cause to different varieties, and also the phylogenetic tree reported by Lebrun and Baudouin (1999) and Baudouin and Ledbrun (2002) that uses microsatellite marker data to show grouping of the most resistant varieties known (MYD, MRD, Malayan Green Dwarf, MXPT2, etc.). These types of studies could be very helpful to select the varieties to be screened, either by traditional field testing for resistance or alternative methods, such as controlled transmission using insect-proof cages like those used by Howard (1995) or in vitro transmission (CICY, Mexico, unpublished). These last two transmission methods would require exposing the plants to be tested to feral insects captured in areas with LY or LYDs. Or even better, would be using insects reared under

controlled conditions and fed with a suspension of phytoplasmas obtained by culturing them in vitro (Contaldo et al. 2016). Altogether, this integrated controlled transmission approach would allow a lot of control of the resistance screening process and more effective and precise results in shorter times. Of course, in order to achieve developing such a transmission system for screening further research is needed to identify vector insects, master rearing of vector insects and culturing in vitro of phytoplasmas causing LY and LYDs. And certainly, resistance to LY or LYDS would be a trait that should be considered in programs for coconut conservation and genetic improvement (Bourdeix 2019; COGENT 2017).

It is also important to undertake research on defence mechanisms in coconut in general. Previous research has shown that benzothiadiazole (BTH), a functional analogue of salicylic acid (SA), could activate the systemic acquired resistance (SAR) mechanism in *Arabidopsis thaliana* (Lawton et al. 1996). Also, that when plants of *A. thaliana* were treated with BTH before inoculation with X-disease phytoplasma, there was reduced infection, and also reduced survival of the phytoplasma vector *Colladonus montanus* when interacting with BHT treated plants (Bressan and Purcell 2005). Then considering that SAR could play a role in phytoplasma defence in plants, a study in coconut evaluated the occurrence of non-expresser of PR genes 1 (NPR1) homologue genes in coconut palm, two were found *CnNPR1* and *CnNPR3* and that the amount of transcripts of both were regulated positively by SA, suggesting that these homologues could be associated with the activation of SAR in coconut (Nic-Matos et al. 2017). It remains to define if this could be relevant for defence as well as the study of other mechanisms of defence in relation to LY and related diseases.

It is also essential to be able to identify resistance or susceptibility to LY and LYDs with faster methods. In a search for disease-resistance gene candidates of the nucleotide binding site (NBS) type from LY resistant or susceptible coconut ecotypes, several DNA sequences were obtained that clustered in seven different clades and their expression changed in response to SA (Puch-Hau et al. 2015). Based on these findings, two markers for susceptibility to LY have been developed and are currently being tested with promising results (CICY, Mexico, unpublished). This type of research should be extended and strengthened in order to identify coconut germplasm that is resistant to LY or LYDs rapidly and to assist other lengthier traditional methods involving vector mediated transmission of phytoplasmas.

What is mentioned in the chapter above addresses LY and related diseases, how to manage them, and the urgent need to identify, in a fast and precise fashion, coconut resistant germplasm for replanting coconut in most producing countries around the world. This is a huge task that would be very difficult to be carried out by seed propagation alone. Because of this pressure, the development of micropropagation protocols has been a priority for several years. Important advances have been obtained with the participation of several countries. In Mexico, a process has been developed that is highly efficient and has been scaled up to commercial level (Oropeza et al. 2016). Nevertheless, further research for improvements are necessary for reducing costs and managing long-distance transportation, among other

things. Again, as mentioned in the above paragraphs, this requires the international collaboration of expert institutions.

Finally, we need to work in a well-organized fashion worldwide to use our resources for research in a more efficient and effective way, and this could be more easily achieved if such an effort is coordinated in collaboration with organizations such as the ICC and COGENT. It is very important to keep in mind that measures for dealing with LY or LYDs are considered within the wider scope of strengthening the coconut value chain in every producing country, but in particular aiming at improving the income and livelihoods of the people working with the coconut palm, mainly the small farmers with lower incomes.

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