Chapter 11
Phytoplasmal Diseases

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Abstract Phytoplasmas are phloem-restricted phytopathogenic mollicutes transmitted by hemipteran insects. Phytoplasmas belonging to different 16S rDNA groups and *Candidatus Phytoplasma* species have been reported to be associated with coconut. Several economically important phytoplasmal diseases affecting coconut have been reported worldwide, which include the lethal yellowing disease in Caribbean and African regions as well as the non-lethal maladies like root (wilt) disease and Weligama leaf wilt in Asia. Application of molecular technologies has enabled the development of phytoplasmal diagnostic techniques. However, molecular detection of coconut phytoplasmas is many a time intriguing owing to its exclusive phloem confinement, non-uniform distribution and subminimal titres in palms. Palm-phytoplasmal interactions as well as transmission mechanism by insects are poorly understood. Since the phytoplasmal diseases cannot be cured, research on incursion management is currently based on strengthening quarantine and breeding for disease resistance. Management practices are also available to obtain satisfactory yield even from disease-affected palms in the case of non-lethal phytoplasmal diseases. Outbreak of phytoplasmal diseases as a victim of climate change is a reality in view of extensive migration and survival superiority of insect vectors even under climate extremities.

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11.1 Introduction

Diseases caused by phytoplasmas pose serious threats to the sustainability of coconut cultivation worldwide. Since the nineteenth century, numerous outbreaks of phytoplasmal diseases leading to the loss of millions of coconut palms have been reported from Asia, Africa and America. Though most of them were recorded in the 1800s, the etiology remained elusive till the discovery of phytoplasma (formerly known as *Mycoplasma*-like organisms, MLOs) by Doi et al. (1967). Most of them were named based on the symptoms or geographic locations. Phytoplasmas are wall-less, pleomorphic, unicellular, nutritionally fastidious, phloem-limited, vector-transmitted phytopathogens with a mean diameter of 200–800 nm and genome size ranging from 530 kb to 1350 kb (Sears and Kirkpatrick 1994; Harrison et al. 2015).

Phytoplasmas constitute a unique monophyletic clade in the class *Mollicutes*. Due to the paucity of distinct phenotypic criteria, currently its classification is based on sequencing and RFLP analysis of the conserved 16S rDNA region (Lee et al. 1998; Martini et al. 2007; Wei et al. 2007). The introduction of molecular tools streamlined the phytoplasma taxonomy and enabled the characterisation and differentiation of these phytopathogens. Phytoplasmas belonging to different taxonomic groups have been reported to be associated with diseases of coconut palms. Some of them are fatal and widespread, whereas others are only debilitating or are limited to certain geographic regions. Phytoplasma diseases of coconut are listed in Table 11.1.

11.2 Lethal Yellowing (LY) and Lethal Yellowing-Type Diseases (LYD)

11.2.1 Occurrence, Distribution and Crop Loss

The most well-known and widespread phytoplasmal disease of coconut is lethal yellowing (LY). The disease was noticed in Cayman Islands in the Caribbean region as early as in 1834, and the first scientific report was from Jamaica in 1891 (Fawcett 1891; Howard 1983; Been 1995). The disease was reported from Cuba in the early 1900s (De La Torre, 1906 quoted by Ntushelo et al. 2013). The presence of the disease in Haiti dates back to the 1920s (Llauger et al. 2002).

From the western end of the island where the disease remained endemic for many years, it moved eastwards across the mountain barrier against prevailing winds to eastern part of the island in the 1960s (Grylls and Hunt 1971). The disease gained epidemic proportions following hurricanes of 1932 and 1933, and by 1982, it had spread over the entire west of the island (Martyn 1949; Nutman and Roberts 1955). LY has been reported from Key West Florida of the USA in 1937 spreading to Miami in 1971 (Martinez and Roberts 1967; Seymour et al. 1972; McCoy 1976), Yucatan Peninsula of Mexico in 1977 (Romney and Harries 1978), Dominican

Table 11.1 Phytoplasma associated with major diseases of coconut, vectors involved and the geographic distribution

Sl. no	Disease	16Sr group	Distribution	Vector	References
1.	Lethal yellowing	16SrIV-A	Florida, Jamaica, Honduras, St. Kitts and Nevis, Cuba	*Haplaxius crudus* (van Duzee)	Harrison et al. (1994a, 2008), Myrie et al. (2006, 2012), Brown et al. (2007) and Howard et al. (1983, 1984)
		16Sr IV-B	Yucatan (Mexico) and Cuba		Tymon et al. (1998)
		16Sr IV- E	Dominican Republic		Martinez et al. (2008)
2.	Lethal yellowing-type diseases				
A	Cape St. Paul wilt disease (CSPWD)	16SrXXII-B	Ghana	*Myndus adiopodoumeensis* Synare and *Diostrombus* spp.[a]	Dabek et al. (1976), Johnson and Harries (1976), Danyo (2011), and Dery et al. (1997b)
B	Kaincope disease (maladie de Kaincopé)	Not characterised	Togo	Not identified	Nienhaus and Steiner (1976) and Dollet and Giannotti (1976)
C	Awka wilt disease (AWD) or bronze leaf wilt	16SrXXII-A	Nigeria	Not identified	Bull (1955), Ekpo and Ojomo (1990) and Osagie et al. (2013, 2016)
D	Kribi disease	Not characterised	Cameroon	Not identified	Dollet et al. (1977)
E	Côte d'Ivoire lethal yellowing (CILY)	16SrXXII-B	Côte d'Ivoire	Not identified	Konan Konan et al. (2013) and Arocha-Rosete et al. (2014, 2015)

(continued)

Table 11.1 (continued)

Sl. no	Disease	16Sr group	Distribution	Vector	References
F	Lethal decline/ Lethal disease (LD)	16SrXXII-A	Mozambique	*Platacantha lutea*[a]	Harrison et al. (2014), Arocha-Rosete et al. (2014) and Dollet et al. (2011)
		16Sr IV-C	Tanzania and Kenya	*Diastrombus mkurangai*, *Meenoplus* spp.[a]	Schuiling and Mpunami (1990), Schuiling et al. (1992a), Nienhaus et al. (1982), Mpunami et al. (1999, 2000) and Bonnot et al. (2010)
G	Bogia coconut syndrome (BCS)	Affinity towards 16Sr IV	Papua New Guinea	*Zophium apupillata*, *Lophopssaccharicida*, Zoraidini species (unidentified), Ricaniidae species (unidentified), *Taparella amata* and *Colgar* sp.[a]	Kelly et al. (2011), Pilotti et al. (2014) and Lu et al. (2016)
3.	Root(wilt) Disease	16Sr XI-B	India	*Proutista moesta* (Westwood), *Stephanitis typica* (Distant)	Solomon et al. (1983), Rajan and Mathen (1985), Mathen et al. (1990) and Manimekalai et al. (2010)
4.	Tatipaka disease	Not characterised	India	Not identified	Rao et al. (1956), Rethinam et al. (1989) and Rajamannar et al. (1993)
5.	Coconut yellow decline	16SrXIV, 16Sr XXXII- B	Malaysia	Not identified	Nejat et al. (2009a)
6.	Kalimantan wilt	16Sr XI and 16Sr XIII	Indonesia	Not identified	Sitepu et al. (1988), Warokka (2005) and Bertaccini et al. (2014)

(continued)

Table 11.1 (continued)

Sl. no	Disease	16Sr group	Distribution	Vector	References
7.	Weligama coconut leaf wilt disease	16SrXI	Sri Lanka	*Goniagnathus (Tropicognathus) punctifer*, *Recilia dorsalis* Motschulsky, *Kolla ceylonica* (Melichar), *Idioscopus clypealis* (Lethierry), *Proutista moesta* (Westwood), *Proutista* sp., *Nisia nervosa* (Motschulsky), *Stephanitis typica* (Distant)[a]	Wijesekara et al. (2008), Perera et al. (2010), Wijesekara et al. (2013) and Kumara et al. (2015)
8.	Blast	Not characterised	Côte d'Ivoire	*Recilia mica* Kramer	Quillec et al. (1978) and Julia (1979)

[a]Not confirmed by vector transmission

Republic (Carter and Suah 1964), New Providence island of Bahamas (Leach 1946; Howard 1983) and Honduras (Ashburner et al. 1996). The disease reached the state of Oaxaca in the Pacific coast of Mexico by 1997 (Harrison et al. 2002a). The disease is currently a matter of concern to coconut cultivation in North and Central America and the Caribbean Basin (Brown et al. 2007).

The name 'lethal yellowing' is often used to refer to yellowing-type diseases of palms in several locations in humid tropics. Though these diseases show similarity in symptoms with the typical LY, they differ in geographical distribution patterns, rates of spread, varietal susceptibility, host range and vector. These diseases are collectively referred to as 'lethal yellowing-type diseases' (LYD). LYD occurring in West Africa include Kaincope disease (maladie de Kaincopé) in Togo (Nienhaus and Steiner 1976; Steiner 1976a), Awka wilt disease (AWD) or bronze leaf wilt in Nigeria (Bull 1955; Ekpo and Ojomo 1990), Kribi disease in Cameroon (Dollet et al. 1977), Cape St. Paul Wilt Disease (CSPWD) in Ghana (Dabek et al. 1976; Johnson and Harries 1976) and lethal yellowing (CILY) in Côte d'Ivoire (Konan Konan et al. 2013; Arocha-Rosete et al. 2014). In East Africa, LYD is referred to as 'lethal decline' (LD) and has been reported from Tanzania (Schuiling and Mpunami 1990 quoted by Mpunami et al. 1999), Mozambique (Mpunami et al. 1999; Bonnot et al. 2010) and Kenya (Nienhaus 1984 quoted by Mpunami et al. 1999). LYD named as Bogia coconut syndrome (BCS) has also been reported from the Oceanian country Papua New Guinea (Kelly et al. 2011).

Devastating losses of coconut plantations due to LY have been recorded since the late nineteenth century. In Jamaica alone, at least 5 million Jamaica Tall palms were killed within 20 years following its spread to the main coconut-growing areas in the east of the island (Romney 1983). The disease caused the death of palms and

reduction in coconut production in Haiti. Within a short span of 15 years (1979–1994), the coconut production declined from 60 million to 30 million nuts a year. The disease killed about 80% of palms in the western region of Haiti (Donis 2002). Losses in coconut production in Belize due to LY were estimated to be 25% in cays and 75% in the main land (Quiroz 2002). During a span of 2 decades from the 1970s to 1990s, lethal yellowing epidemics decimated most of the Jamaican Tall variety in Florida and Jamaica and spread to neighbouring regions including the Pacific coast of the Americas (Harrison et al. 2002a). The destructive disease caused the death of about 90% of the palms in the Honduran Atlantic coast and Jamaica (Doyle 2001; Myrie 2002). The disease caused the destruction of 650,000 palms in Yucatan Peninsula and 100,000 coconut palms in Florida (Mora-Aguilera 2002).

LYD has devastated millions of palms across several countries in Africa. CSPWD has almost destroyed the coconut industry in the Volta region and has wiped out 5500 ha of coconut palms in south western Ghana putting about 90% of the country's coconut at risk (Ofori and Nkansah-Poku 1997). Over a period of 30 years, 'lethal decline' destroyed about 56% palms in southern Tanzania (Mpunami et al. 1999), and CSPWD has annihilated about 1 million coconut palms in Ghana (Nipah et al. 2007). In Nigeria, 'Awka wilt' killed over 98% of the West African Tall (WAT) palms in 11 years (Odewale et al. 2010).

11.2.2 Symptoms

The pattern of appearance and sequential progression of disease symptoms usually vary based on the phytoplasma group, geographical location, host species and variety (Dollet et al. 2009; Harrison et al. 2014). The first visual symptom on infected bearing coconut palms is the premature nut fall/fruit drop. Most of the fallen nuts have brown to black water-soaked regions under the calyx. This is followed by blackening/necrosis of newly emerging inflorescences. Most of the male flowers will be dead, and there will not be any fruit set in the affected inflorescences. As the inflorescence necrosis phase progresses, yellowing of the leaves starts from the leaves in the outer whorls, advancing upwards, affecting the younger, middle and finally the upper leaves. The yellow leaves turn brown and dry and die. The dried leaves remain hanging on the crown for a few days before falling. Eventually, the whole crown perishes, leaving a bare trunk or 'telephone pole'. The estimated time lag from initial infection by the pathogen to the appearance of first symptom has been variously reported as follows: in mature bearing palms, 230–450 days (Romney 1972) and 210–450 days (Heinze et al. 1972) and for young nonbearing palms, at least 240–270 days (Dabek 1974). The time between probable initial infection and death of mature palms has been reported as 3–6 months (Grylls and Hunt 1971) or 4–5 months (McCoy et al. 1983). In addition to these symptoms in above ground parts, death of root tips coincides with the foliar yellowing symptom. Roots also show necrosis, which becomes more extensive as the disease progresses (Eden-Green 1979).

Though symptoms of LYD in West Africa and Tanzania are similar to LY in the American continent (Mpunami et al. 1999), some differences are noticed. While in Kaincope and SPWD, the spear is often found to be dead with many fronds still remaining green; in lethal yellowing, the spear leaf appears unaffected except for some necrotic patches (Johnson and Harries 1976). In some LY-affected palms, the spear leaf or a mid-crown leaf occasionally shows yellowing prematurely (McCoy et al. 1983). Sometimes inflorescence necrosis becomes conspicuous only after the appearance of frond yellowing as reported in Guatemala (Mejia et al. 2004). Unlike LYD, inflorescence necrosis is absent in BCS reported from Papua New Guinea (Kelly et al. 2011).

11.2.3 Aetiology

Preliminary aetiological investigations were focussed on the role of bacteria, fungi, virus, viroids and other abiotic factors. Despite the severity of economic losses and intensive research, the cause of LY and LYDs remained as a mystery till the 1960s. The discovery of the association of phytoplasmas with plant diseases during the 1960s sparked the search for phytoplasmas in LY-affected palms. In 1972, three groups independently reported the occurrence of phytoplasma in the phloem of coconut palms showing LY symptoms by transmission electron microscopy (Beakbane et al. 1972; Heinze et al. 1972; Plavsic-Banjac et al. 1972). Electron microscopic observations of LY-diseased coconut palms showed that phytoplasma was present in partly expanded inflorescences and spear leaves, but not found in fully expanded inflorescences, leaves or stems (Parthasarathy 1974).

The physical presence of phytoplasma in tissue from spear leaf and unopened inflorescences of coconut palms affected with Kaincope disease in Togo and Cape St. Paul wilt in Ghana was also confirmed by transmission electron microscopy (Dabek et al. 1976; Dabek 1977). Association of phytoplasma with LD in East African countries has also been established by fluorescence microscopy using the fluorochromes DAPI (Deutsch and Nienhaus 1983).

A cause-effect relationship between phytoplasmas and the disease was established by the differential response of LY- and LYD-affected palms to antibiotics. LY palms treated with penicillin showed no recovery response, whereas symptom remission occurred when they were treated with oxytetracycline (Hunt et al. 1974; McCoy 1972). Remission of symptoms in palms after treatment with tetracycline further supported the phytoplasmal etiology of Kaincope disease in Togo and lethal disease in Tanzania, Kenya and Mozambique (Steiner 1976b; Kaiza 1987).

The advent of molecular techniques has enabled systematic identification and differentiation of different phytoplasmas. The taxonomic classification of phytoplasma is now based on the highly conserved genes coding for the 16S rRNA and the spacer region between 16S and 23S rRNA (Lee et al. 1993; Schneider et al. 1993). Though the similarity in symptoms initially supported a common etiology for LY and LYDs, the differences in epidemiology and coconut ecotype susceptibility led to the specu-

lation that coconut-associated phytoplasmas in Africa are probably distinct from those affecting palms in the Caribbean Basin (Schuiling et al. 1992a, b). Restriction fragment length polymorphism (RFLP) profiles of the PCR amplification product also showed differences in restriction pattern indicating that the phytoplasma of LY in the Caribbean and East and West Africa are of different strains (Harrison et al. 1994b). Studies on phytoplasma associated with LY-diseased palms in the Americas have shown genetic differences between the LY phytoplasmas in Florida, México and Jamaica based on the analysis of rDNA (Harrison et al. 2002b) and between Cuba and Mexico based on the analysis of non-ribosomal DNA (Llauger et al. 2002).

Phytoplasmas associated with LY and LYD of palms are most commonly placed in the 16SrIV group although some have now been reclassified into group 16SrXXII (Lee et al. 1998; Harrison et al. 2014). The 16SrIV group is subdivided into A–F reflecting the genetic variation, host range and vectors (Harrison et al. 2002a, b; Brown et al. 2006; Harrison et al. 2008; Martinez et al. 2008; Vázquez-Euán et al. 2011). The subgroups 16SrIV-A, 16SrIV-B, 16SrIV-C and 16SrIV-E are pathogenic to coconut palms. The phytoplasma-causing LY to coconut palms in Florida, Jamaica, Honduras and Nevis falls in 16SrIV-A (Myrie et al. 2006; Brown et al. 2007; Harrison et al. 2008), whereas those causing LY in Mexican Yucatan and Cuba are placed in 16SrIV-B subgroup (Tymon et al. 1998; Llauger et al. 2002). The phytoplasma associated with lethal disease of coconut palms in Kenya and Tanzania belongs to 16Sr IV-C (Tymon et al. 1998). The subgroup 16Sr IV-E has been reported to be associated with LY of palms in southern coast of Dominican Republic (Martinez et al. 2008). The partial 16S rDNA sequence of phytoplasma associated with BCS in Papua New Guinea matched most closely (96%) with that of group 16SrIV (Kelly et al. 2011). Oropeza et al. (2011) analysed the distribution of phytoplasma in LY-affected palms using PCR, and pathogen titre was found to be high in the stem, young leaves, inflorescences, stem apex and root apex when compared to intermediate leaves and roots without apex.

Studies on the LYD pathogen in East Africa using 16S rRNA gene sequence and RFLP analysis showed that phytoplasma associated with Mozambique LYD is distinct from that of Kenya and Tanzania but closely related to those from West Africa, CSPWD in Ghana and AWD in Nigeria (Mpunami et al. 1999). Virtual RFLP profiles of the F2n/R2 portion of the 16S rRNA gene and pattern similarity coefficients revealed that the phytoplasma strains associated with coconut LYD in Mozambique, Nigeria, Ghana and Côte d'Ivoire represent a distinct species-level lineage and delineated into a novel taxon, *Candidatus Phytoplasma palmicola* (16SrXXII). Phytoplasma associated with LYD in Mozambique and Nigeria belongs to 16SrXXII-A, and those causing diseases in Ghana and Côte d'Ivoire are placed in 16Sr XXII-B subgroup (Arocha-Rosete et al. 2014; Harrison et al. 2014). It is also common that diverse groups of phytoplasmas can occur in a geographic region. The association of 3 different groups, viz. West African subgroup (XXII-B), East African subgroup (IV-C) and a novel unclassified *Candidatus Phytoplasma pini*-related strain with LYD in Mozambique, supports this theory (Bila et al. 2015a, b).

11.2.4 Transmission

With the establishment of phytoplasmal etiology of LY and LYD, the search for insect vectors narrowed down to Auchenorrhyncha insects of the order Hemiptera, i.e. leafhopper and plant hopper. The most definitive method for determining the vector species within a given pathosystem is a transmission test. But this approach for coconut has several logistical constraints, viz. difficulties involved in the caging and maintenance of the perennial and woody palms during the incubation period, collection and release of large number of vectors that have acquired phytoplasma, etc. Though conventional vector transmission trials for LY in palms have been carried out since the 1960s, very few were successful (Tsai 1980; Eden-Green 1995). To date, the only established vector of LY is the plant hopper *Haplaxius crudus* (van Duzee) (formerly *Myndus crudus*) in Florida (Howard et al. 1983).

Several insects have been reported as putative vectors of LY and LYD based on PCR results. However, their vector status has not yet been established by cage transmission trials. There have been a great number of unsuccessful palm phytoplasma vector transmission trials. In Ghana, studies on putative insect vectors of CSPWD were initiated in 1990 (Philippe et al. 2009). Even though *Myndus adiopodoumeensis* Synare (Homoptera: Cixiidae) and *Diostrombus* spp. (Homoptera: Derbidae) tested positive for CSPWD phytoplasma in PCR, these hoppers failed to elicit the disease in subsequent cage transmission trials. In spite of the extensive transmission studies conducted, the vector of CSPWD in Ghana remains unknown (Danyo 2011).

An alternate method that helps to surpass the use of caged palms in the preliminary screening trials is the feeding medium approach (Tanne et al. 2001). The method involves assaying phytoplasma DNA in the sucrose solution, on which the insect feeds, through a parafilm barrier. Even though this technique does not provide a conclusive proof of vector status, it is advantageous as the detection of phytoplasma DNA in the feeding media by PCR indicates the competency of the insect to introduce phytoplasma during feeding activity. Lu et al. (2016) combined single-insect feeding medium tests with loop-mediated isothermal amplification (LAMP) to identify putative vectors of BCS.

The possibility of transmission of phytoplasma through seed nuts from diseased palms has been regarded as highly unlikely due to the phloem-limited nature of the pathogen. However, Harrison and Oropeza (1997) reported the presence of lethal yellowing phytoplasma in at least one seed from nuts of 3 coconut palms manifesting primary stage symptoms of LY using pathogen-specific PCR. DNA of the LY phytoplasma was detected in 18.06% of embryos from fruits of 4 diseased Atlantic Tall coconut palms by PCR assays (Cordova et al. 2003). Nipah et al. (2007) detected CSPWD phytoplasma in 9 out of the 52 embryos from diseased West African Tall (WAT) palms by PCR employing phytoplasma universal primer pair P1/P7 nested with CSPWD-specific primer pair G813f/AwkaSR. Since the embryo lacks direct connection with sieve elements, the mechanism of transmission remains

unclear. Oropeza et al. (2017) demonstrated for the first time using nested PCR and TaqMan real-time PCR the occurrence of LY phytoplasma transmission from coconut embryos to plantlets obtained by *in vitro* plumule culture. However, there is no conclusive evidence, so far, for the presence of intact phytoplasma in the embryos and seedlings raised from nuts of LY- and LYD-affected palms.

11.2.5 Detection

LY-diseased palms are traditionally identified by symptoms, electron microscopy or fluorescence microscopy. Accumulation of DNA in extra-nuclear sites indicative of phytoplasmal infection could be demonstrated by staining the tissues with DAPI (4′,6′-diamidino-2-phenylindole), a fluorochrome that binds to DNA and fluoresces under UV radiation (Russel et al. 1975). Although the microscopic techniques are useful for establishment of association of phytoplasma with the disease, it cannot differentiate one phytoplasma from another. On the other hand, procedures of electron microscopy for the phytoplasmal detection are time-consuming. Very low concentration of the pathogen in coconut palm species and uneven distribution within vascular tissues make its detection difficult. The advent of molecular techniques has made identification and differentiation of different phytoplasmas comparatively easier. Harrison et al. (1992) have developed DNA probes for detection of phytoplasma of Caribbean origin. Similarly, oligonucleotide primer for selective amplification of LY phytoplasma by DNA polymerase chain reaction (PCR) has been standardised (Rhode et al. 1993; Harrison et al. 1994a; Mpunami et al. 1997; Tymon and Jones 1997).

Spear leaves from around the apical meristem, which is rich in phloem, are the most reliable source of phytoplasma detection in palms (Harrison et al. 1999). Often the old palms are tall and sample collection from the crown is difficult. Trunk shavings obtained by drilling a hole 10–15 cm into the trunk is a non-destructive and less laborious method of sample extraction for successful phytoplasma detection (Harrison et al. 2002b). Myrie et al. (2011) developed improved methods, viz. a multiplex direct-PCR system, PCR on 16S rRNA and hemolysin genes; a SYBRGreen system based on the *GroEL* gene; and real-time PCR using TaqMan probes based on the 16S rDNA and *GroEL* gene for detection of LY phytoplasma-affected palms in Jamaica. These methods with improved sensitivity and specificity together with quantisation capability enhance the efficiency of detection. Córdova et al. (2014) developed a TaqMan real-time PCR assay for detection and quantification of LY phytoplasmas belonging to 16SrIV A, D and E subgroups in America. For the detection of CSPWD, sec A gene-based PCR assay was developed by Yankey et al. (2014).

11.2.6 Disease Management

The non-availability of technologies for the curative treatment of LY and LYD accentuates the need for periodic surveillance and removal of affected palms to prevent the spread of the disease. Black's approach, pioneered by a Jamaican farmer Michael Black, involves an integration of on-farm quarantine, strict weekly surveillance, cutting down and burning of palms with disease symptoms and replanting with a variety selected for high yield and disease resistance as well as whole-farm weed control, and a good fertilisation regime significantly reduced the disease incidence over years (Myrie et al. 2011; Gurr et al. 2016). The removal of infected palms slowed down the disease spread in Dominican Republic and Ghana (Martinez et al. 2008, 2010; Nkansah et al. 2009).

Though oxytetracycline antibiotics have been shown to reduce phytoplasma infection in palms (McCoy et al. 1976), they have never been considered as a management strategy in view of the prohibitive costs and health hazards. Management of the vectors by employing various techniques like insecticide spraying, replacing the ground cover with non-host grass species, mulching and mass trapping are not considered as sustainable management strategies (Howard and Oropeza 1998).

Use of resistant varieties is the best option for management of a phytoplasmal disease. The Jamaica Tall grown in Jamaica and Caribbean is highly susceptible to LY. In Jamaica, field testing for LY resistance of coconut germplasm was carried out in 6 resistance trials planted from 1961 to 1970. Among the cultivars, the Jamaican Tall/Atlantic Tall (AT) was the most susceptible, with 90% mortality. The Sri Lankan, Indian and Malayan Dwarfs and the King Coconut appeared to be highly resistant to LY with less than 5% mortality. However, in some locations in Florida and Jamaica, the susceptibility of Malayan Dwarf ranged between 42.9–100% and 14–40%, respectively (Howard et al. 1987). Though Malayan Dwarfs possessed high degree of resistance to the LY, its poor productivity under marginal conditions hindered the adoption by farmers. Experiments were conducted in Jamaica during the 1970s for the production of a hybrid with high disease resistance and productivity. The Panama Tall, with an intermediate mortality of 44%, was used as pollen donor in a programme to produce hybrid palms. The 'Maypan' hybrid (Malayan Dwarf × Panama Tall), combining the higher resistance of the Dwarf cultivar with large size and adaptability of the Tall, has not only the advantage of the hybrid vigour but also was found to be only 10% susceptible to LY. The Maypan has formed a good compromise between resistance level, yield and product quality. The faster growth rate coupled with greater adaptability to different habitats and poor soils is the added advantage of Maypan over the Malayan Dwarf. After devastating losses of the local Jamaican Tall variety to LYD, the Malayan Dwarf varieties and Maypan became the primary foci of coconut replanting programmes, which led to a recovery of the coconut industry (Been 1995; Ashburner and Been 1997; Harrison et al.

2002a). High mortality rate of MYD and Maypan planted in LY-infected regions in recent years indicate that these cultivars cannot be considered resistant to LY as previously thought. In a coconut cultivar trial at Fort Lauderdale, Florida, Malayan Dwarfs and Maypan hybrids showed a high degree of susceptibility of 70% and 83%, respectively (Broschat et al. 2002). Massive destruction of Malayan Dwarfs and its hybrid by LYD triggered research exploring the possibility of the genetic contamination of parents, variation or mutation of pathogen/vector that altered the host-vector-pathogen interactions. Research on this line provided evidences on genetic contamination in Panama Tall and MYD in Jamaica. This might have caused some loss of resistance but is insufficient to explain a massive outbreak of the disease (Baudouin et al. 2008; Lebrun et al. 2008). In a trial established in 1991 on the northern coast of Yucatan, Tall and Dwarf populations representing the genetic diversity in Mexico were exposed to LY for 15 years. The result of this experiment indicates that together with MYD, coconut populations from the Pacific coasts of Mexico could be an important source of germplasm to deal with LY (Zizumbo-Villarreal et al. 2008). It is imperative to explore populations, other than Malayan Dwarf, with good resistance to broaden the genetic base.

The Malayan Dwarfs which showed resistance to LY in Jamaica were found to be susceptible to CSPWD in Ghana. The high CSPWD resistance shown by the Sri Lankan Green Dwarf (SGD) and Vanuatu Tall (VTT) varieties in the Dixcove trials (1981–1983) led to the development of the SGD × VTT hybrid which is being used for replanting (Mariau et al. 1996; Dery et al. 1997a; Quaicoe et al. 2009). The good agronomic characteristics of SGD × VTT coupled with its resistance to CSPWD make it a promising hybrid to revamp the coconut industry in Ghana (Dare et al. 2010). Field resistance of Green Dwarfs to AWD in Nigeria was reported by Odewale et al. (2006).

11.3 Blast

Blast disease of coconut was first noticed from Côte d'Ivoire in 1971 (Quillec et al. 1978). The disease generally occurs along with another disease, dry bud rot, noticed on nursery seedlings and in juvenile palms. The symptoms are identical to what is noticed in the blast disease in oil palm in Côte d'Ivoire. The characteristic symptoms are wet rot of spear and roots, yellow brown colouring of the bole and rapid drying of the plant starting with the oldest leaves. The disease occurs mainly in the first year of planting and is rarely noticed in older plants. The disease could be transmitted to periwinkle, which exhibited wilting and yellowing symptoms (Dollet 1979). EM examination of symptomatic periwinkle revealed intraphloemic phytoplasma. Tetracycline experiment further confirmed phytoplasmal etiology.

The disease is transmitted by *Recilia mica* (Jassidae, Deltocephalinae) (Julia 1979). The insect preferably multiplies in grasses. Spraying with Temik reduced blast incidence to the extent of 40%. Shading and phytosanitation such as clean

weeding in and around nursery seedlings help to keep the insect population low. Growing cover crop such as *Pueraria* suppresses the growth of the grasses thereby reducing the vector population in the field.

11.4 Root (Wilt) Disease

11.4.1 Occurrence, Distribution and Crop Loss

Root (wilt) is one of the major devastating diseases affecting coconut palms in India. The occurrence of the disease was first noticed as early as in 1874 in the erstwhile state of Travancore (present Kerala state, India). The disease became conspicuous after the great floods of 1882. The disease was initially reported from 3 independent locations each at a distance of about 50 km (Butler 1908; Pillai 1911; Varghese 1934). It is a major production constraint in southern districts of Kerala, creating serious economic distress to the agrarian families. According to a survey made during 1984–1985, the disease occurs in a contiguous manner in an area of 4,10,000 ha in 8 out of the 14 districts of the Kerala state starting from Thiruvananthapuram district in the south extending up to Thrissur district and in isolated pockets in the remaining 6 northern districts. The crop loss due to the disease was estimated to be about 968 million nuts (Anon 1985). The total estimated monetary loss in terms of loss in husk, copra yield and leaf number and quality of leaves on the basis of 1984 price index for coconut was of the order of about Rs.3000 million. As per the sample survey conducted in 1996, the disease intensity in the contiguous diseased tract ranged between 2.1% in Thiruvananthapuram district and 48.0% in Alappuzha district, and there was an overall reduction in disease incidence from 32.37 to 24.05. This reduction is attributed to the removal of about 6 million diseased palms, replanting with quality seedlings, adoption of disease management practices and crop conversion from coconut to rubber (Anon 1996). The disease has also been reported from the bordering districts of Tamil Nadu, Karnataka and Goa (Solomon et al. 1999; Chandramohanan 2010). It is a continuing threat to the coconut palms in India.

The disease is non-lethal but debilitating and palms of all age groups are affected. Disease contraction in the pre-bearing age delays flowering and affects the vitality of the palm (Ramadasan et al. 1971). About 65% of the root (wilt)-diseased palms are affected by a fungal disease known as leaf rot (Srinivasan 1991). The leaf rot disease superimposed on root (wilt) diseased palms leads to rapid decline and reduction in yield (Menon and Nair 1948, 1951). The disease occurs in all major soil types (Menon 1938), but the spread is faster in sandy, sandy loam, alluvial and in heavy textured soils than in laterites. Relatively higher incidence is recorded in waterlogged low-lying areas adjacent to rivers and canals (Pillai et al. 1973). The pattern of spread is erratic, occurs in jumps or leaps, characteristic of insect transmission (Pillai et al. 1980).

11.4.2 Symptoms

The most obvious diagnostic symptom of the disease is the abnormal bending of the leaflets termed 'ribbing' or 'flaccidity'. Foliar yellowing of the outer whorl of leaves and marginal necrosis are the other associated symptoms (Radha and Lal 1972) (Fig. 11.1).

In seedlings and juvenile palms, yellowing of foliage is virtually absent, and flaccidity is the only conspicuous symptom. Instance of diseased palms having green outer leaves, with yellowing of leaves in some of the inner whorls, is also noticed (Menon 1938). With the progress of the disease, extensive rotting of the roots is observed. The main roots and rootlets start drying from the tip backwards (Menon and Nair 1951). Shedding of immature nuts is yet another symptom observed in some cases. Drying up of spathe and necrosis of spikelets from the tip downwards in unopened inflorescence are noticed in certain cases (Maramorosch 1964). A high percentage of pollen produced is either sterile or less viable (Varkey and Davis 1960). Meiotic irregularities are also observed (Nambiar and Prasannakumari 1964). The vitality of the diseased palms is so adversely affected that they produce small spathes with fewer female flowers. In the advanced stage, the crown gets very much reduced in size and ceases to produce inflorescence (Menon 1938). The nuts from diseased palms have thinner husk, and fibres are definitely weaker and less firm (Varghese 1934). The kernel is thinner and never dries up but remains soft and flexible. The tender coconut water of diseased palm is insipid. The oil content is very much reduced and the oil loses its flavour as well (Menon 1938).

Fig. 11.1 Root (wilt) disease of coconut super imposed with leaf rot. Note the symptoms such as flaccidity, foliar yellowing and necrosis. (Photo: V. Krishnakumar)

An indexing method for quantifying the disease intensity giving due weightage to the 3 predominant foliar symptoms has been developed (George and Radha1973). Disease index, $\mathrm{DI} = \sum \frac{F+Y+N}{L} \times 10$, where F (0–5), Y (0–3) and N(0–2) are the grade points assigned to flaccidity, yellowing, necrosis and L, is the total number of leaves in the palm. For indexing young palms below 10 years, more weightage is to be given to flaccidity as it is found to be the prominent symptom and disease index $\left(\mathrm{DI} = \sum \frac{15F+5Y+5N}{L} \times 10 \right)$. Disease index can vary from 0 to 100,where 0 represents the total absence of all the symptoms, indicating that the palm is visually disease-free (apparently healthy) and 100 means the presence of all the symptoms in the acute stage on all the leaves. Based on the disease index, the palms can be categorised into disease early (DI < 20), disease middle (DI 20–50) or disease advanced (DI > 50). The indexing method was further simplified by Nambiar and Pillai (1985) by scoring the symptoms on the leaves present in any of the 5 spirals. Usually, only 4–5 leaves are present in a spiral, and hence, the number of observations per palm to be taken for calculation of disease index is less. The indexing system helps in quantifying disease severity in a simple numerical expression that can be analysed statistically.

Comparative anatomical studies of the tender unopened leaves of healthy and root (wilt) affected palms revealed general stunting of epidermal cells, reduction in thickness of the cuticle on the adaxial side and differential rate of division of the upper epidermis in leaves of diseased palms. Reduction in wall thickness of cells, sclerenchymatous fibres and bundle sheath is also observed. These anatomical changes may be contributing to the downward curling of leaflets. In diseased palms, wall thickness of metaxylem elements is reduced resulting in uneven shape of the xylem component. The uptake and translocation of solutes are also impeded with the formation of tyloses in xylem vessels as well as gummosis and necrotic obliteration of phloem in the roots of diseased palms (Govindankutty 1981). The distribution of stomata per unit area is found to be more in leaflets of diseased palms (Joseph and Shanta 1964). The stomatal regulation is also adversely affected due to the disease. The stomata in diseased palms fail to close in response to soil and atmospheric drought (Rajagopal et al. 1986a, b). The overall disturbance in absorption and transpiration may also be contributing to the flaccidity symptoms.

11.4.3 Aetiology

Ever since its report in the 1880s, the causal agent of RWD remained as an enigma for a century. Preliminary investigations on the aetiology of the disease were primarily focused on fungal pathogens associated with the root rot of coconut.

Physiological and biochemical changes observed in diseased palms such as derangement in uptake and translocation of solutes, higher respiration and transpiration rate, altered nitrogen metabolism and an accelerated phenol metabolism are indicative of a pathogen-mediated aberrant host metabolism than of a physiological disorder (Rajagopal et al. 1998). Soil sickness as a contributing factor of the disease was excluded based on field fertility trials as well as tissue and soil analysis (Cecil and Amma 1991). The sporadic occurrence and spreading nature of the disease implied the involvement of a pathogen as the cause of the disease.

Etiological investigations gained new dimensions with the advancement in plant pathology. The role of fungi, bacteria, virus, viroid and nematodes in inducing the disease was ruled out based on the results of the inoculation studies and pathogenicity experiments (Nagaraj et al. 1954; Shanta and Menon 1959; Summanwar et al. 1969; Maramorosch and Kondo 1977; Randles and Hatta 1980; Joseph and Lilly 1991; Jayasankar and George 1991; Sosamma and Koshy 1991). Electron microscopic examination of juvenile tissues like sub-meristem, petiole of developing leaves, rachilla of unopened inflorescence and root tips of diseased palms revealed the presence of phytoplasma (Solomon et al. 1983). These pleomorphic phloem-bound mollicutes are observed only in sieve tubes. The size of the coccoid forms ranged from 250 nm to 400 nm. Distribution of the phytoplasma within the vascular bundle is rather sparse, and not all sieve elements in a patch contained them. In the older leaves, moribund forms, lacking internal contents, only were observed. Constant association of phytoplasma with the disease has been conclusively established with the finding of the organism in more than 75 diseased palms and their absence in an equal number of healthy palms studied (Solomon et al. 1999).

Light microscopic studies with Diene's staining and fluorescent microscopy with 4,6-diamidino-2 phenylindole 2 HCI (DAPI) or Hoechst 33258 lent support to the association of phytoplasma in the phloem tissues of RWD affected palms (Solomon et al. 1987). The results of the experiments on antibiotic therapy (Pillai and Raju 1985; Chowdappa et al. 1989), dodder transmission (Sasikala et al. 1988) and vector transmission (Mathen et al. 1990) provided conclusive evidences on the association of phytoplasma with RWD. Molecular characterisation of 16S rRNA sequences of the RWD pathogen added further evidence to the phytoplasmal etiology of the disease. The phytoplasma-causing RWD has been characterised as *Candidatus Phytoplasma oryzae*-related strain belonging to 16SrXI-B subgroup (rice yellow dwarf group) (Manimekalai et al. 2010, 2014a).

11.4.4 Transmission

Phytoplasmas are generally transmitted by insects belonging to the Homoptera group. A plant hopper, *Proutista moesta*, and a leaf hopper, *Sophonia greeni,* both belonging to the Homopteran group, have been consistently found in coconut foliage besides lace bug, *Stephanitis typica* (Heteroptera:Tingidae). Acquisition of phytoplasma by these putative insect vectors while feeding on diseased palm was

studied. Phytoplasma was observed in the salivary glands of lace bug 18–23 days after feeding on diseased palms (Mathen et al. 1987) and also in plant hoppers fed on diseased palms for over 30 days (Rajan et al. 2002). The vector role of lace bug and plant hopper has been conclusively established in transmission experiments (Mathen et al. 1990; Rajan 2011).

Field experiments were conducted to assess the control of aerial insects on fresh incidence of disease. The results were not encouraging. This may be due to the perennial nature of the crop, the presence of insect vector throughout the year, the persistent mode of transmission of phytoplasma and the reinfestation of sprayed plants within a short period (Solomon et al. 1999). The phytoplasma also could be experimentally transmitted from coconut to periwinkle, an indicator host, through dodder laurel (*Cassytha filiformis*). Periwinkle grown in insect-proof cages bridged to diseased coconut palms through dodder laurel developed chlorotic spots in the interveinal areas and at vein endings of fully opened leaves in 3–4 weeks of haustorial establishment. Detection of phytoplasma in periwinkle, dodder laurel and the source palm confirmed transmission of the disease (Sasikala et al. 1988). Though Manimekalai et al. (2014b) confirmed the presence of DNA of root (wilt) phytoplasma in 16.67% embryos of nuts collected from diseased palms, the potential of seed transmission of phytoplasma was checked by subjecting the germinated seedlings raised from mature nuts collected from diseased palms. But phytoplasma DNA could not be detected in any of the raised seedlings.

11.4.5 Diagnosis

Preliminary investigations on the development of diagnostic techniques were based on the biochemical tests of altered host metabolisms detectable in the form of either accumulation or depletion of substances consequent to differential enzymatic activity in diseased palms (Joseph and Shanta 1963; Lal 1968; Dwivedi et al. 1977). As these changes can also be induced by other biotic and abiotic stresses, more thrust was given on the development of a rapid and reliable diagnostic techniques. Physiological changes like stomatal resistance and transpiration rate in healthy and diseased palms were also used for diagnosis of RWD. High stomatal resistance with a correspondingly low transpiration rate was recorded in healthy palms in contrast to low stomatal resistance and high transpiration rate in diseased palms (Rajagopal et al. 1986a). With the establishment of the phytoplasmal aetiology, protocols were standardised for the purification of RWD phytoplasma (Mayil Vaganan et al. 2001), production of polyclonal antisera specific to coconut RWD phytoplasma and direct antigen coated-enzyme linked immunosorbent assay (DAC-ELISA) for the detection of RWD phytoplasma even 24 months before symptom manifestation (Sasikala et al. 1998, 2001, 2004, 2010).

With the advent of molecular biology, efforts were made to develop nucleic acid-based detection techniques for RWD phytoplasma. Preliminary attempts made to detect the coconut RWD phytoplasma using 16S rRNA sequence-based universal

primers failed to give consistent results in direct and nested PCRs. Molecular detection of phytoplasma associated with RWD was achieved by modification of phytoplasma enrichment technique for DNA extraction by addition of 5% polyvinylpyrrolidone, designing 6 highly sensitive primers and semi-nested PCR technique (Manimekalai et al. 2010; Ramaswamy et al. 2016). A real-time PCR protocol was also developed for detection of RWD phytoplasma (Manimekalai et al. 2011). However, it was observed that there was no consistency in detection of phytoplasma by nested PCR, real-time PCR and LAMP when large number of root (wilt) affected coconut samples were tested, and further refinement of these techniques are necessary for reliable and rapid detection of RWD in the early stage of infection (Hegde et al. 2016).

11.4.6 Breeding for Resistance

Since there are no prophylactic or curative measures available for managing the RWD, an enduring solution to the problem lies in breeding varieties resistant to the disease. Systematic evaluation trials at ICAR-CPCRI have led to the release of 2 coconut varieties and 1 hybrid for cultivation in the root (wilt) disease-prevalent areas. Purity of D × T hybrid seedlings is assessed using SSR markers (Krishnakumar et al. 2015). For details please see Chap. 5 on varietal resistance.

11.4.7 Disease Management

A noticeable feature of the disease is that it is not lethal but debilitating. Field experiments were conducted to assess the possibility of preventing the fresh incidence of the disease through control of aerial insects. The results were not encouraging. This may be due to the perennial nature of the crop, the presence of insect vector throughout the year, the persistent mode of transmission of phytoplasma and their infestation on sprayed palms within a short period (Solomon et al. 1999). Though Pillai et al. (1991) reported remission of symptoms by OTC treated palms, it cannot be recommended either as a prophylactic or curative measure as the antibiotic has to be given repetitively and the cure is temporary. Prohibitive cost of the antibiotic and the caution required in its use for treating plant diseases are the other factors which weigh against it being recommended as a treatment.

Since there are no measures available to control the disease to perfection, coconut farmers have to ‘live with the disease’, and certain integrated disease management practices are advocated to ensure a satisfactory yield even in a disease-prevalent area (Sahasranaman et al. 1983, Amma et al. 1983; Bavappa et al. 1986; Muralidharan et al. 1991; Krishnakumar et al. 2015).

1. Removal of palms: All disease advanced and uneconomic palms with annual yield of less than 10 nuts are to be removed.
2. Replanting: Replanting with disease-resistant varieties or elite seedlings from high-yielding disease-free palms located in heavily disease-affected tracts.
3. Biopriming: Biopriming of seedlings with *Pseudomonas fluorescens* to impart tolerance.
4. Application of organic manures: Application of 25 kg farmyard manure or 10 kg vermicompost enriched with *Trichoderma harzianum* at 100 g.
5. Biomass recycling: Application of leguminous green manure crops and *Gliricidia* leaves.
6. Fertiliser application: Application of recommended dose of fertilisers (500g N, 300 g P_2O_5,1250 K_2O and 250 g $MgSO_4$ $palm^{-1}year^{-1}$) in two splits.
7. Liming: Application of lime/dolomite supplemented with magnesium sulphate.
8. Irrigation: Irrigation with 250 l of water $palm^{-1}week^{-1}$, soil moisture conservation and providing adequate drainage wherever necessary.
9. Intercropping and farming system: Raise intercrops in rotation, adopting mixed cropping/mixed farming coupled with recycling of organic matter.
10. Adopting recommended management strategies for leaf rot disease, rhinoceros beetle and red palm weevil.

11.5 Tatipaka Disease

11.5.1 Occurrence, Distribution and Crop Loss

Tatipaka is a slow debilitating phytoplasmal disease of coconut in India. Its distribution is confined to East and West Godavari, Srikakulam, Nellore, Krishna and Guntur districts of Andhra Pradesh state in India. The disease was first noticed by the farmers of Tatipaka village after the cyclone of 1949, and the disease was named after the village from where it was observed (Rao et al. 1956). However, the reports of survey conducted during 1956–1958 in Srikakulam district indicated the presence of the disease 20 years prior to the cyclone (Subbiah and Rao 1963; Pandit et al. 1969; Rethinam et al. 1989). The disease generally occurs in heavy black deltaic soils than in sandy, sandy loam and red loam soils. It is observed in both well-managed and neglected gardens. A survey made during 1985–1990 in the central delta of the river Godavari 'Konaseema' (which accounts for 60% of the area under coconut in the state) revealed that the disease is prevalent in 85 out of 201 villages (Narasimhachari et al. 1991). Altogether, 8179 palms were identified as Tatipaka diseased by field surveys (Rajamannar et al. 1993).

The disease is non-lethal but of a debilitating nature, generally affecting palms in the age groups of 20–60 years. Palms below 20 years are very rarely affected

(Rethinam et al. 1989). The spread of the disease is not contiguous but sporadic at a slow pace of 3.5% over a period of 5 years. Later surveys revealed that the disease incidence in the field is less than 1% (Srinivasulu et al. 2006).

11.5.2 Symptoms

The disease-affected palms generally bear profusely for 2–3 years before the expression of visual symptoms, and more number of dark leaves (often fasciated) appear in the crown. With the onset of disease, there is a reduction in both number and size of leaves. The leaves exhibit characteristic chlorotic water-soaked spots, and the fronds bend abnormally, sometimes twisting in loops. In the advanced stage with the narrowing of leaflets and reduction in size of crown, the affected palm looks like a date palm. The spathes produced are very small with very few rachillae. The bunches carry a mixture of normal and atrophied nuts. The atrophied nuts are barren with thinner spongy mesocarp with or without shell, copra and nut water. The undersized nuts show longitudinal cracks with occasional gumming. In the advanced stage of the disease, the stem tapers and produces smaller spathes and inflorescences, which ultimately do not bear any fruit. Reduction in number and size of the roots and extensive rotting of roots are the characteristic underground symptoms of the disease (Rao 1966; Ramapandu and Rajamannar 1981).

11.5.3 Aetiology

The disease was listed as one of the uncertain aetiologies till the 1990s. The involvement of fungi and bacteria in the incidence of the disease was conclusively ruled out as these microbes could not be consistently isolated from diseased palms. Sap transmission and purification studies have also indicated the non-viral nature of the disease (Ramapandu and Rajamannar 1981; Rajamannar et al. 1984). Polyacrylamide gel electrophoretic analysis of isolated nucleic acid from diseased palms did not confirm the association of any viroid-type pathogen (Randles and Hatta 1980).

Remission of symptoms observed in diseased palms treated with tetracycline hydrochloride indicated the association of a phloem-limited phytoplasma with the disease (Rajamannar et al. 1993). This finding was corroborated by light microscopy using Diene's staining, a fluorescent microscopy with aniline blue as fluorochrome and antibiotic therapy (Rajamannar et al. 1994). Electron microscopic examination of spindle, leaves and rachillae of unopened inflorescences from diseased palms revealed the presence of phytoplasma in sieve tubes further confirming the aetiology of the disease (Srinivasulu et al. 2006).

11.5.4 Disease Management

Attempts to control the disease by foliar and soil application of various chemicals, hormones and nutrients did not yield any encouraging result. Root regeneration of affected palms also could not ameliorate the condition of palms (Rajamannar et al. 1993). Since there are no prophylactic or curative measures available for treating phytoplasmal diseases, the options left are to arrest the spread of disease by systematic surveillance and rouging of diseased palms as and when identified and using disease-resistant planting material. The programme of identifying the diseased palms and eradication is a more practical step as the disease is confined to a limited geographical region, the number of diseased palms is comparatively less and the spread is reported to be very slow. The programme, if systematically implemented, will help in total eradication of the disease. Survey of the heavily diseased area indicated the cultivar, Gangabondam, to be free of the disease, whereas the incidence was high in East Coast Tall (Rajamannar et al. 1994). This observation has to be confirmed so that this cultivar could be used as a parent in the future breeding programmes for evolving disease-resistant varieties.

11.6 Coconut Yellow Decline (CYD)

CYD is a debilitating disease that reduces the productivity of coconut in Malaysia.

11.6.1 Occurrence, Distribution and Crop Loss

Coconut lethal yellowing-like symptoms were first reported from Malaysia by Sharples (1928). The disease was not widespread in Malaysia till the end of the twentieth century. However, Nejat et al. (2009a) observed typical symptoms of phytoplasmal infection, viz. yellowing and drying of fronds, in Malayan Dwarf ecotypes in coconut plantations in Selangor State in Malaysia. On the basis of disease symptoms, this infectious disease has been named Coconut Yellow Decline (CYD).

11.6.2 Symptoms

Yellowing of leaves in the outer whorl, which eventually turn light brown, is the characteristic preliminary symptom of the disease. The yellowing spreads rapidly to the younger leaves, and ultimately the emerging spear leaves become chlorotic. The

affected palms also show premature nut fall and inflorescence necrosis. As the disease advances, gradual collapse of fronds and terminal rot of the growing point of immature palms occur. Affected palms generally die within 5 months after the appearance of initial symptoms (Nejat et al. 2009a).

11.6.3 Aetiology

CYD in Malaysia is reported to be caused by 2 different groups of phytoplasma. Based on sequences of 16S rDNA, the CYD isolates found in Malayan Red Dwarf (MRD) and Malayan Tall (MT) palms at Serdang were found to belong to the Bermuda grass white leaf group (16SrXIV, *Candidatus Phytoplasma cynodontis* group), while those from Malayan Yellow Dwarf (MYD) palms at Banting formed a novel group *Candidatus Phytoplasma malaysianum* (16Sr XXXII-B) (Nejat et al. 2009a, b). Both 16SrXIV and 16Sr XXXII-B groups causing CYD exist in Selangor State of Malaysia, reiterating the theory of occurrence of diverse groups of phytoplasma in a geographic region. The whole transcriptome profile of MRD in response to infection by 16SrXIV *Candidatus Phytoplasma cynodontis* group was evaluated by Nejat et al. (2015) using RNA-Seq technique. The transcriptomes' profile indicated that out of the 39,783 differentially expressed unigenes, 21,860 were downregulated and 18,013 were upregulated following phytoplasma infection.

11.6.4 Detection

Nejat et al. (2010) developed a real-time PCR assay using a 16S rDNA-based TaqMan primer–probe set, for sensitive, quantitative and rapid detection of the 16Sr XXXII-B of CYD phytoplasmas. The technique developed could not detect the 16SrXIV phytoplasma associated with CYD, and hence, it can be used to distinguish between infections caused by the two different CYD phytoplasmas present in Malaysia. Though remarkable developments have been achieved in the genomics of the pathogen, the vector involved in the transmission of the disease still remains unknown.

11.7 Kalimantan Wilt

11.7.1 Occurrence, Distribution and Crop Loss

Kalimantan wilt (KW) is a major phytopathological constraint that has caused extensive damage to coconut plantations in Central Kalimantan of Indonesia. The occurrence of the disease was initially noticed by farmers in 1978, and an outbreak

of the disease was reported in 1988 in East Kotawaringin and Kapuas in Central Kalimantan (Sitepu et al. 1988). Based on the survey conducted in 1997, it was reported that the disease affects more than 100,000 palms in Kecamatan Mentaya Hilir Selatan and Kecamatan Pulau Hanaut. Coconut cultivation occupies a prime position in the agrarian economy of Indonesia. The loss of palms due to this phytoplasmal disease resulted in the economic upheaval of thousands of farm families in the country.

11.7.2 Symptoms

The first visible diagnostic symptom of the disease is the wilting and drying of older fronds. The petioles of the dried leaves break near the base and hang down, skirting the trunk. Blackening and rotting of the young developing inflorescences are another characteristic symptom. Older inflorescences may be normal or partially rotten. In newly opened inflorescences, rotting or blackening is seen on the middle but not on the tip as seen in LY-affected palms. Another commonly observed symptom is the presence of brown streaks along the petiole and midribs of green fronds. The affected palm dies within a period of 4 months after the appearance of the initial symptom (Warokka 1998).

11.7.3 Aetiology

The pathogenic role of bacteria, fungi, viruses, viroids and nematodes in inducing the KW symptoms could not be established (Warokka 1998). The phytoplasmal aetiology of Kalimantan wilt was confirmed by using nested PCR (Warokka et al. 2006). Based on the 16S rRNA sequences, the phytoplasmas causing KW have been reported to belong to 16Sr XI and 16Sr XIII groups (Warokka 2005 quoted by Bertaccini et al. 2014).

11.8 Weligama Coconut Leaf Wilt Diseases (WCLWD)

11.8.1 Occurrence and Distribution

Weligama coconut leaf wilt disease (WCLWD) was first reported in 2006 from the Weligama Divisional Secretariat Division (DSD) in the Matara district in southern Sri Lanka. The disease is now prevalent in Matara, Galle and Hambantota districts (Wijesekara et al. 2008; Perera et al. 2010; Everard 2013).

11.8.2 Symptoms

The symptoms of WCLWD are akin to that of root (wilt) disease reported from India. The earliest and characteristic diagnostic symptom of the disease is the flattening and downward bending of leaflets giving the frond a ribbed or flaccid appearance. In the initial stage of the disease, the crown of affected palms appears dark green in colour. The degree of flaccidity of leaflets varies among the fronds in a single palm. Intense yellowing of outer whorls of leaves is also a specific symptom of the disease. The yellowing becomes more prominent just after the rainy season. Flaccidity of leaflets is evident on seedlings younger than 3 years, whereas yellowing is seen only in older palms. Occasionally, yellowing of 6 to 8 fronds in the middle whorl is observed in affected palms. Yellowing is followed by drying up of the leaflets that starts from the margins of the affected fronds, and dried fronds hang in the crown for some time before falling off. The fronds also curl downward giving a ragged appearance to the crown. In some palms, the tips of fronds get twisted, break and hang down. Unopened spear leaves lose their rigidity and bend downwards in severely affected palms. Due to reduction in the number of fronds, the crown appears smaller and the trunk begins to taper (Fig. 11.2).

Below ground symptoms include the high degree of necrosis of root tips in moderately affected palms and in palms with high disease severity; no young roots are observed due to root degeneration. The roots of the affected palms show intense branching followed by necrosis of the stellar region of young white roots

Fig. 11.2 Weligama coconut leaf wilt disease. (Photo: GV Thomas)

(Wijesekara et al. 2008). As the disease progresses, female flower production declines and the palm becomes unproductive. Palms of any stage are susceptible to the disease, and palms over 3 years are most commonly affected. Furthermore, like RWD, WCLWD also predisposes the palms to a fungal disease complex called leaf rot disease. Weerakkody (2010) reported 40–60% reduction in yield in the advanced stages of the disease. Weerakoon et al. (2011) evaluated the impact of WCLWD on morphology, physiology and yield of coconut palms and found that the disease causes significant yield reduction. The whole nut weight of the palms diminishes with the severity of the disease. Though the copra yield is reduced by the WLCWD, the oil content is not affected much.

Waidyarathne and Samarasinghe (2014) developed artificial neural networks to assess naturally existing WCLWD severity status using data mining approaches. Attempts were made to develop methods to detect diseased coconut palms with prominent foliar yellowing symptom using multispectral satellite images of 0.5 m resolution. Adult coconut palms in the advanced stages of the disease can be detected by this method with more than 80% accuracy. But this method is not suitable for the precise detection of the disease in seedlings and in early stages of the disease. The overall accuracy of this approach is only 60–70% (Nainanayake et al. 2016a).

11.8.3 Aetiology

Analysis of leaflets from WCLWD-affected palms grown in fertilised lands revealed that the yellowing is not related to nutrient deficiency, particularly, magnesium, potassium and calcium (Wijesekara et al. 2008). The similarity of symptoms to the RWD and the symptom remission in oxytetracycline-treated palms supported the hypothesis of phytoplasmal etiology. Hence, the DNA extracted from midribs of spear leaves was subjected to nested PCR with phytoplasma universal primers R16F2n/R16R2 and R16mF2/R16R2 nested with fU5/rU3, P1/P7 nested with Chrfor/rU3 and direct PCR with Pc399/P1694. PCR products of expected sizes were obtained from diseased but not from healthy palms from a disease-free area. The sequences generated from the PCR products were submitted to similarity search (BlastN) in the NCBI database which confirmed that a phytoplasma belonging to the 16SrXI *Candidatus Phytoplasma oryzae* group is associated with WCLWD. The phytoplasma was found to be 99% similar to sugarcane white leaf phytoplasma, sugarcane grassy shoot phytoplasma and RWD phytoplasma, but not identical (Perera et al. 2010, 2012; Wijesekara et al. 2013).

11.8.4 Transmission

The confirmation of the phytoplasmal etiology instigated the research on vectors transmitting the disease. Kumara et al. (2015) collected 32 homopteran and a few hemipteran species from WCLWD-affected coconut plantations and subjected to

the nested PCR using universal phytoplasma-specific primers, P1/P7 and Pc399/P1694. Eight homopteran species, viz. *Goniagnathus (T.) punctifer*, *Recilia dorsalis* Motschulsky, *Kolla ceylonica* (Melichar), *Idioscopus clypealis* (Lethierry), *Proutista moesta* (Westwood), *Proutista* sp., *Nisia nervosa* (Motschulsky) and an unknown Cixiid and a hemipteran species, *Stephanitis typica* (Distant), gave positive bands at 1280 bp which on sequencing showed similarity to WCLWD phytoplasma sequence (Gene Bank: EU635503), suggesting them as putative vectors of WCLWD. The vectoral role of these insects has to be confirmed by transmission experiments.

11.8.5 Detection

Concerted efforts are being made to develop serological techniques for the detection of WCLWD. The antibodies developed for the serological detection by using purified antigen from WLCD-affected spear leaves were found to be not specific to the pathogen, and therefore, production of monoclonal antibodies was initiated (Wijesekara et al. 2013). Detection of WLCWD presently depends on PCR amplification of DNA using phytoplasma-specific primers and characterisation of amplicon by sequencing. As the phytoplasma is present in very low titer in WCLWD-affected coconut palms, phytoplasma enrichment procedure is followed for DNA extraction. Siriwardhana et al. (2012) reported a DNAzol direct DNA extraction protocol followed by LAMP colorimetric assay for the detection of phytoplasma associated with WCLWD. The protocol has to be validated with more number of samples and needs further refinement before field application.

11.8.6 Management

Since the disease is confined to the Southern Province only, the Coconut Research Institute (CRI) of Sri Lanka decided to adopt intensive measures to prevent its possible spread to the rest of the country. Accordingly, a 3-km-wide and 80-km-long buffer zone was identified demarcating the diseased area. This buffer zone is inspected for the occurrence of the disease. The diseased and uneconomic palms are removed to curtail further spread. The spread of the disease was further checked by prohibiting the transportation of any palm species and their live parts out of the demarcated boundary. Findings of preliminary experiments conducted in the affected area have shown that the spread of the disease could be effectively contained by these measures (Nainanayake et al. 2013). However, isolated incidences in some places quite far from the boundary are being reported (Nainanayake et al. 2016b).

Research work on breeding for resistance was also initiated to identify tolerant varieties. Sri Lanka Green Dwarf is identified as a promising cultivar with a very

high degree of resistance to WCLWD. The *Nana* coconut also shows a fair degree of resistance, though not as high as that of Sri Lanka Green Dwarf (Perera et al. 2015). SLGD is being used for resistance breeding programmes for the production of planting material for replanting in WCLWD endemic areas.

11.9 Future Strategy

Phytoplasmal diseases continue to be a serious threat to the coconut cultivation as they are non-curable. The difficulty in culturing the phytoplasma under axenic conditions remains a bottleneck in unveiling the mechanisms involved in pathogenesis. Despite the advancements in phytoplasma genomics, the information on the molecular basis of disease development on coconut palms is sparse. Recent developments in molecular biology should be fully utilised for elucidating the complex biological processes involved in the palm-phytoplasma-vector interactions. The knowledge on the effect of climate change on vector dynamics and disease spread is a prerequisite to formulate future strategies to mitigate the impact of phytoplasmal diseases. It is imperative to contain the spread of the disease within the current geographical limits by appropriate quarantine measures. Periodic surveillance in the diseased tract and monitoring for new incidence of disease and prompt removal will go a long way in arresting fresh outbreaks. The best option to control phytoplasma diseases of course is evolving disease-resistant/disease-tolerant planting material and hence deserves priority attention.

References

Amma PGK, Cecil RS, Pillai NG, Mathew AS, Nambiar PTN (1983) Performance of Dwarf × Tall hybrid coconut in root (wilt) affected areas of Kerala under different fertilizer levels. In: Bavappa KVA, Iyer RD, Das PK, Sethuraj MR, Ranganathan V, Nambiar KKN (eds) Proceedings of PLACROSYM V. Indian Society for Plantation Crops, Kasaragod, pp 405–410

Anonymous (1985) Coconut root (wilt) disease. Intensity, production loss and future strategy. CPCRI, Kasaragod, 45

Anonymous (1996) Coconut root (Wilt) survey. Department of Agriculture, Government of Kerala, Thiruvananthapuram, p 22

Arocha-Rosete Y, Konan Konan JL, Diallo AH, Allou K, Scott JA (2014) Identification and molecular characterization of the phytoplasma associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire. Can J Plant Pathol 36:141–150

Arocha-Rosete Y, Konan-Konan JL, Diallo AH, Allou K, Scott J (2015) Analyses based on the 16S rRNA and secA genes identify a new phytoplasma subgroup associated with a lethal yellowing-type disease of coconut in Côte d'Ivoire. Phytopathol Mol 5:S57

Ashburner GR, Been BO (1997) Characterization of resistance to lethal yellowing in *Cocos nucifera* and implications for genetic improvement of this species in the Caribbean region. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like diseases of coconut. Natural Resources Institute, Chathman, pp 173–183

Ashburner G, Cardova I, Oropeza C, Illingworth R, Harrison N (1996) First report of coconut lethal yellowing disease in Honduras. Plant Dis 80:960

Baudouin L, Lebrun P, Berger A, Myrie W, Been B, Dollet M (2008) The Panama Tall and the Maypan hybrid coconut in Jamaica: did genetic contamination cause a loss of resistance to Lethal Yellowing? Euphytica 161:353–360

Bavappa KVA, Jayasanker NP, Radha K, Rethinam P (1986) Coconut root (wilt) disease, Present status of research and management. Technical bulletin no. 14, CPCRI, Kasaragod, p 10

Beakbane A, Clater C, Posnette A (1972) Mycoplasmas in the phloem of coconut, *Cocos nucifera* L. with lethal yellowing disease. J Hort Sci Biotech 47:265

Been BO (1995) Integrated pest management for the control of lethal yellowing quarantine, cultural practices and optimal use of hybrids. In: Oropeza C, Howard FW, Ashburner GR (eds) Lethal yellowing research and practical aspects. Kluwer Academic Publishers, Dordrecht, pp 101–109

Bertaccini A, Duduk B, Paltrinieri S, Contaldo N (2014) Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. American J Plant Sci 5:1763–1788

Bila J, Mondjana A, Samils B, Högberg N (2015a) High diversity, expanding populations and purifying selection in phytoplasmas causing coconut lethal yellowing in Mozambique. Plant Pathol 64:597–604

Bila J, Mondjana A, Samils B, Högberg N (2015b) Potential novel '*Candidatus* Phytoplasma pini'-related strain associated with coconut lethal yellowing in Mozambique. Phytopathol 5:S59

Bonnot F, de Franqueville H, Lourenço E (2010) Spatial and spatiotemporal pattern analysis of coconut lethal yellowing in Mozambique. Phytopathol 100:300–312

Broschat TK, Harrison NA, Donselman H (2002) Losses to lethal yellowing cast doubt on coconut cultivar resistance. Palms 46:185–189

Brown SE, Been BO, McLaughlin WA (2006) Detection and Variability of the Lethal Yellowing Group (16SrIV) Phytoplasma in the *Cedusa* sp. (Hemiptera: Auchenorrhyncha: Derbidae) in Jamaica. Ann Appl Bio 149:53–62

Brown SE, Been BO, McLaughlin WA (2007) The lethal yellowing (16Sr IV) group of phytoplasmas. Pest Technol 1(1):61–69

Bull RA (1955) Bronze leaf wilt of coconut palms in Nigeria. J West African Inst Palm Res 3:70–72

Butler EJ (1908) Report on coconut palm disease in Travancore. Agric Res Inst Pusa Bull (9):23

Carter W, Suah JRR (1964) Studies on the spread of lethal yellowing disease of the coconut palm. FAO Plant Prot Bull 12:73–78

Cecil SR, Amma PGK (1991) Soils and nutrition. In: Nair MK et al (eds) Coconut root (wilt) disease, Monograph series no.3. CPCRI, Kasaragod, pp 45–52

Chandramohanan R (2010) Symptomatology and etiology. In: Thomas GV, Chandramohanan R, Jacob PM, Krishnakumar V (eds) Coconut root (wilt) disease management. Central Plantation Crops Research Institute, Kasaragod, pp 1–18

Chowdappa P, Gopinathan Pillai N, Gunasekaran M, Solomon JJ (1989) Distribution and retention of Oxytetracycline hydrochloride injected into coconut palms affected by root(wilt) disease. J Plantn Crops 17(2):108–113

Cordova I, Jones P, Harrison NA, Oropeza C (2003) *In situ* PCR detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing disease. Mol Plant Pathol 4(2):99–108

Córdova I, Oropeza C, Puch-Hau C, Harrison N, Collí-Rodríguez A, Narvaez M, Nic-Matos G, Reyes C, Sáenz L, Edizioni ETS (2014) A real-time PCR assay for detection of coconut lethal yellowing phytoplasmas of group 16SrIV subgroups A, D and E found in the Americas. J Plant Pathol 96:343–352

Dabek AJ (1974) Biochemistry of coconut palms affected with the lethal yellowing disease in Jamaica. J Phytopathol 81:346–353

Dabek AJ (1977) Electron microscopy of Kaincope and Cape St. Paul Wilt diseased coconut tissue from West Africa. Phytopathol 38:341–346

Dabek AI, Johnson CG, Harries HC (1976) Mycoplasma-like organisms associated with Kaincope and Cape St. Paul Wilt diseases of coconut palms in West Africa. PANS 22(3):354–358

Danyo G (2011) Review of scientific research into the Cape Saint Paul Wilt Disease (CSPWD) of coconut in Ghana. African J Agric Res 6:4567–4578
Dare D, Andoh-Mensah E, Owusu-Nipah J, Yankey N, Quaicoe RN, Nkansah-Poku J, Dery SK (2010) Evaluation of some basic traits of a promising coconut hybrid: Srilankan Green Dwarf crossed to Vanuatu Tall (SGD × VTT). J Sci Technol 30(3):9–14
Dery SK, N'Cho YP, Sangare A, Arkhurst ED (1997a) Cape St Paul wilt disease: resistance screening and prospects for rehabilitating the coconut industry in Ghana. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like disease of coconut, Elmina, Ghana. Natural Resources Institute, Chatham, pp 147–151
Dery SK, Philippe R, Mariau D (1997b) Preliminary observations Auchenorrhyncha (Homoptera): suspected vectors of St. Paul wilt disease in Ghana. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like disease of coconut. Natural Resources Institute, Chatham, pp 269–276
Deutsch E, Nienhaus F (1983) Further studies on distribution of mycoplasma like organisms in different tissues of lethal diseased coconut palms in Tanzania. Z Pflkrankh Pjlschutz 90:278–284
Doi Y, Teranaka M, Yora K, Asuyama H (1967) Mycoplasma or PLT Group like microorganisms found in the phloem elements of plants infected with Mulberry Dwarf, Potato Witches' Broom, Aster Yellows or Paulownia Witches' Broom. Jpn J Phytopathol 33:259–266
Dollet M (1979) Research on the etiology of blast of oil and coconut palms. In: Thomas DL, Howard FW, Donselman HM (eds) Proceedings of the fourth meeting of the international council on Lethal Yellowing. Pub. FL-80-1. University of Florida, Fort Lauderdale, p 19
Dollet M, Giannotti J (1976) Kaincope disease: presence of mycoplasma in the phloem of diseased coconuts. Oleagineux 31:169–171
Dollet M, Giannotti J, Renard JL, Ghosh SK (1977) Study of lethal yellowing of coconuts in Cameroon: Kribi disease. Observations of mycoplasma like organisms. Oleagineux 32:317–322
Dollet M, Quaicoe R, Pilet F (2009) Review of coconut "Lethal Yellowing" type diseases diversity, variability and diagnosis. Oléagineux 16:97–101
Dollet M, Macome F, Vaz A, Fabre S (2011) Phytoplasmas identical to coconut lethal yellowing phytoplasmas from Zambesia (Mozambique) found in a pentatomide bug in Cabo Delgado province. Bull Insectol 64:S139–S140
Donis J (2002) Coconut cultivation and lethal yellowing status in Haiti. In: Proceedings of expert consultation on sustainable coconut production through control of lethal yellowing disease. CFC/FAO/CIB, Kingston, pp 90–92
Doyle MMR (2001) Crisis research: managing lethal yellowing disease. Biotechnol Dev Monit 44/45:12–15
Dwivedi RS, Mathew C, Ray PK, Amma BSK, Ninan S (1977) Rapid biochemical test to detect root (wilt) disease of coconut. Curr Sci 46:611–612
Eden-Green SJ (1979) Root symptoms in coconut palms affected by lethal yellowing disease in Jamaica. FAO Plant Prot Bull 4:119–122
Eden-Green SJ (1995) A brief history of lethal yellowing research. In: Oropeza C, Howard FW, Ashburner GR (eds) Lethal yellowing: research and practical aspects. Kluwer Academic Publishers, Dordrecht, pp 17–33
Ekpo EN, Ojomo EE (1990) The spread of lethal coconut diseases in West Africa: incidence of Awka disease (or bronze leaf wilt) in Ishan area of Bendel State of Nigeria. Principes 34:143–146
Everard JMDT (2013) Integrated management of Weligama coconut leaf wilt disease program in Sri Lanka: strategies, implementation and challenges. In: Gunasena HPM, Gunathilake HAJ, Fernando LCP, Everard MDT, Appuhamy PAHN (eds) Weligama coconut leaf wilt disease six years after. Coconut Research Institute, Lunuwila, pp 13–17
Fawcett W (1891) Report on the coconut disease at Montego Bay. Bull Bot Dept (Jamaica) 23:2
George MV, Radha K (1973) Computation of disease index of root (wilt) disease of coconut. Indian J Agric Sci 43:366–370

Govindankutty MP (1981) Histopathological studies. In: Review of research on coconut root (wilt) disease. Central Plantation Crops Research Institute, Regional Station, Kayamkulam, pp 49–54

Grylls NE, Hunt P (1971) A review of the study of the aetiology of coconut lethal yellowing disease. Oleagineux 26:311–315

Gurr GM, Johnson A, Ash G, Wilson B, Ero M, Pilotti C, Dewhurst C, You M (2016) Coconut lethal yellowing diseases: a phytoplasma threat to palms of global economic and social significance. Front Plant Sci 7:5121

Harrison NA, Oropeza C (1997) Recent studies on detection of lethal yellowing disease phytoplasmas in the Americas. In: Eden-Green SJ, Ofori F (eds) Proceedings of an international workshop on lethal yellowing-like diseases of coconut. Natural Resources Institute, Chatham, pp 221–234

Harrison NA, Bourne CM, Cox RL, Tsai JH, Richardson P (1992) DNA probes for detection of mycoplasma-like organisms associated with lethal yellowing of palms in Florida. Phytopathol 82:216–224

Harrison NA, Richardson PA, Kramer JB, Tsai IH (1994a) Detection of the mycoplasma-like organisms associated with lethal yellowing of palms in Florida by Polymerase Chain Reaction. Plant Pathol 43:998–1008

Harrison NA, Richardson P, Jones P, Tymon A, Eden Green S, Mpunami A (1994b) Comparative investigation of MLO's associated with Caribbean and African coconut lethal decline diseases by DNA Hybridization and PCR Assays. Plant Dis 78:507–511

Harrison N, Cordova I, Richardson P, Dibonito R (1999) Detection and diagnosis of lethal yellowing. In: Oropeza C, Verdeil JL, Ashburner GR, Cardeña R, Santamaría JM (eds) Current advances in coconut biotechnology, vol 35. Springer, Dordrecht, pp 183–196

Harrison NA, Narváez M, Almeyda H, Cordova I, Carpio ML, Oropeza C (2002a) First report of group 16SrIV phytoplasmas infecting coconut palms with leaf yellowing symptoms on the Pacific coast of Mexico. Plant Pathol 51(6):808

Harrison NA, Myrie W, Jones P, Carpio ML, Castillo M, Doyle MM, Oropeza C (2002b) 16S rRNA interoperon sequence heterogeneity distinguishes strain populations of palm lethal yellowing phytoplasma in the Caribbean region. Ann Appl Biol 141:183–193

Harrison NA, Helmick EE, Elliott ML (2008) Lethal yellowing-type diseases of palms associated with phytoplasmas newly identified in Florida, USA. Ann Appl Biol 153:85–94

Harrison NA, Davis RE, Oropeza C, Helmick EE, Narváez M, Eden-Green S, Dollet M, Dickson M (2014) '*Candidatus* Phytoplasma palmicola', associated with a lethal yellowing-type disease of coconut (*Cocos nucifera* L.) in Mozambique. Intl J Syst Evolut Microbiol 64:1890–1899

Harrison NA, Gundersen-Rindal D, Davis RE (2015) "*Candidatus* Phytoplasma". Bergey's Manual of Systematics of Archaea and Bacteria 1–33

Hegde V, Nair S, Gangaraj KP, Merin Babu (2016) PCR and LAMP based detection of phytoplasma associated with root (wilt) disease of coconut and yellow leaf disease of arecanut. In: Paper presented in 6th international conference: plant, pathogens and people, 23–27 February, 2016, New Delhi, Abstract, p 160

Heinze KG, Petzold H, Martwitz R (1972) Beitrug Zur Atiologie der Todichen vergilbung der Kokospalme. Phytopatholgische Zeitschrift 74:230–237

Howard FW (1983) World distribution and possible geographic origin of palm lethal yellowing disease and its vectors. FAO Plant Prot Bull 31:101–113

Howard FW, Oropeza C (1998) Organic mulch as a factor in the nymphal habitat of Myndus crudus (Hemiptera: Auchenorrhyncha: Cixiidae). Florida Entomol 81:92–97

Howard FW, Norris R, Thomas D (1983) Evidence of transmission of palm lethal yellowing agent by a plant hopper, *Myndus crudus* (Homoptera, Cixiidae). Trop Agric 60:168–171

Howard FW, Williams DS, Norris RC (1984) Insect transmission of lethal yellowing to young palms. Intl J Entomol 26:331–338

Howard FW, Atilano R, Barrant CI, Harrison NA, Theobold WF, Williams DS (1987) Unusually high lethal yellowing disease incidence in Malayan Dwarf coconut palms on localized sites in Jamaica and Florida. J Plantn Crops 15:86–100

Hunt P, Dabek AI, Schuiling M (1974) Remission of symptoms following tetracycline treatment of lethal yellowing affected coconut palms. Phytopathol 64:307–312

Jayasankar NP, George M (1991) Etiology – B. Bacteria. In: Coconut root (wilt) Disease, Monograph series no.3. Central Plantation Crops Research Institute, Kasaragod, pp 17-18

Johnson CG, Harries HC (1976) A survey of Cape St. Paul wilt of coconut in West Africa. Ghana J Agric Sci 9:125–129

Joseph T, Lilly VG (1991) Etiology – A. Fungi. In: Coconut root (wilt) disease, Monograph series no.3. Central Plantation Crops Research Institute, Kasaragod, pp 13–15

Joseph T, Shanta P (1963) Studies on a colour test for the root (wilt) disease of coconut. Abstracts of 50th Indian Sci. Cong Pt III, pp 618–619

Joseph T, Shanta P (1964) Anatomical changes in the tissues of the tender leaves of coconut palms affected by the root (wilt) disease. J Indian Bot Soc 42:61–65

Julia JF (1979) Mise en evidence et identification des insectes responsables des maladies juveniles du cocotier et du palmier a huile en Cote d' Ivoire. Oleagineux 34:385–393

Kaiza DA (1987) History and etiology of the lethal disease of coconut palm (*Cocos nucifera L.*) in Tanzania. M.Sc. thesis, Sokoine University of Agriculture, Tanzania

Kelly PL, Reeder R, Kokoa P, Arocha Y, Nixon T, Fox A (2011) First report of a phytoplasma identified in coconut palms (*Cocos nucifera*) with lethal yellowing-like symptoms in Papua New Guinea. New Dis Rep 23:9

Konan Konan JL, Allou K, Atta Diallo H, Saraka Yao D, Koua B, Kouassi N, Benabid R, Michelutti R, Scott J, Arocha-Rosete Y (2013) First report on the molecular identification of the phytoplasma associated with a lethal yellowing-type disease of coconut palms in Cote d' Ivoire. New Dis Rep 28:3

Krishnakumar V, Babu M, Thomas RJ, Josephrajakumar, Anithakumari P, Hegde V, Chowdappa P (2015) Root (wilt) disease of coconut- Bench to Bunch strategies. ICAR-CPCRI Tech Bull 91:28

Kumara ADNT, Perera L, Meegahakumbura MK, Aratchige NS, Fernando LCP (2015) Identification of putative vectors of weligama coconut leaf wilt disease in Sri Lanka. In: Chakravarthy AK (ed) New horizons in insect science: towards sustainable pest management. Springer, Delhi, pp 137–146

Lal SB (1968) Root (wilt) disease. Resume of work done since 1964. In: Proceedings of 3rd session of the FAO Tech Working Party on Coconut Production, Protection and Processing, Jakarta, Indonesia, pp 9–19

Leach R (1946) The unknown disease of coconut palm in Jamaica. Trop Agric (Trinidad) 43:59–68

Lebrun P, Baudouin L, Myrie W, Berger A, Dollet M (2008) Recent lethal yellowing outbreak: why is the Malayan Yellow Dwarf coconut no longer resistant in Jamaica? Tree Genet Genome 4(1):125–131

Lee IM, Hammond RW, Davis RE, Gundersen DE (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma like organisms. Phytopathol 83:834–842

Lee IM, Gundersen-Rindal DE, Bertaccini A (1998) Phytoplasma: ecology and genomic diversity. Phytopathol 88:1359–1366

Llauger R, Becker D, Cueto J, Peralta E, González V, Rodríguez M, Rohde W (2002) Detection and molecular characterization of phytoplasma associated with lethal yellowing disease of coconut palms in Cuba. J Phytopathol 150:390–395

Lu H, Wilson BAL, Ash GJ, Woruba SB, Fletcher MJ, You M, Yang G, Gurr GM (2016) Determining putative vectors of the Bogia Coconut Syndrome phytoplasma using loop-mediated isothermal amplification of single-insect feeding media. Sci Rep 6:35801

Manimekalai R, Soumya VP, Kumar RS, Selvarajan R, Reddy K, Thomas GV, Sasikala M, Rajeev G, Baranwal VK (2010) Molecular detection of 16SrXI group phytoplasma associated with root (wilt) disease of coconut (*Cocos nucifera*) in India. Plant Dis 94:636

Manimekalai R, Nair S, Soumya V, Roshna O, Thomas G (2011) Real-time PCR technique-based detection of coconut root (wilt) phytoplasma. Curr Sci 101(9):1209–1213

Manimekalai R, Soumya VP, Nair S, Thomas GV, Baranwal V (2014a) Molecular characterization identifies 16SrXI-B group phytoplasma (‘*Candidatus Phytoplasma oryzae’*-related strain) associated with root wilt disease of coconut in India. Sci Hort 165:288–294

Manimekalai R, Nair S, Soumya VP (2014b) Evidence of 16SrXI group phytoplasma DNA in embryos of root wilt diseased coconut palms. Aust Plant Pathol 43:93–96

Maramorosch K (1964) A survey of coconut diseases of unknown etiology. FAO, Rome, p 39

Maramorosch K, Kondo F (1977) Electron Microscopy of leaf sections from Kerala wilt diseased coconut palm. J Plantn Crops 5:20–22

Mariau D, Dery SK, Sangare A, N’Cho YP, Philippe R (1996) Coconut lethal yellowing diseaseand planting material tolerance. Plant Rech Dev 3:105–112

Martinez AP, Roberts DA (1967) Lethal yellowing of coconut in Florida. Proc Fla State Hortic Soc 80:432–436

Martinez RT, Narvaez M, Fabre S, Harrison N, Oropeza C, Dollet M (2008) Coconut lethal yellowing on the southern coast of the Dominican Republic is associated with a new 16SrIV group phytoplasma. Plant Pathol 57:366–366

Martinez RT, Baudouin L, Berger A, Dollet M (2010) Characterization of the genetic diversity of the Tall coconut (*Cocos nucifera* L.) in the Dominican republic using microsatellite (SSR) markers. Tree Gene Genome 6:73–81

Martini M, Lee IM, Bottner KD, Zhao Y, Botti S, Bertaccini A, Harrison NA, Carraro L, Marcone C, Khan AJ, Osler R (2007) Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. Intl J Syst Evol Microbiol 57:2037–2051

Martyn EB (1949) Further observation on the unknown disease of coconut. Trop Agric (Trinidad) 26:110–112

Mathen K, Solomon JJ, Rajan P, Geetha L (1987) Electron microscopic evidence on the role of *Stephanitis typica* (Distant) as vector of coconut root (wilt) disease. Curr Sci 56:1339–1341

Mathen K, Rajan P, Radhaknshnan Nair CP, Sasikala M, Gunasekaran M, Govindankutty MP, Solomon JJ (1990) Transmission of root (wilt) disease to coconut seedlings through *Stephanitis typica* (Distant) (Heteroptera; Tingidae). Trop Agric (Trinidad) 67(1):69–73

Mayil Vaganan M, Alka G, Chithra KR (2001) Biochemical evidences for purification of phytoplasma of root (wilt) disease of coconut. J Plant Biol 28:55–60

McCoy RE (1972) Remission of lethal yellowing in coconut palm treated with tetracycline antibiotics. Plant Dis Rep 56:1019–1021

McCoy RE (1976) Comparative epidemiology of the lethal yellowing, Kaincope, and cadang-cadang diseases of coconut palm. Plant Dis Rep 60:498–502

McCoy RE, Carroll VJ, Poucher CP, Gwin GH (1976) Field control of coconut lethal yellowing with oxytetracycline-hydrochloride. Phytopathol 66:1148–1150

McCoy RE, Howard FW, Tsai JH, Donselman HM, Thomas DL, Basham HG, Atilano RA, Eskafi FM, Britt L, Collins ME (1983) Lethal yellowing of palms, Agri Expt Station Bull No. 834. University of Florida, IFAS, Gainesville

Mejia F, Palmieri M, Oropeza C, Doyle M, Harrison N, Aguilar E, Narváez M, Estrada R, Ortiz G (2004) First report of coconut lethal yellowing disease in Guatemala. Plant Pathol 53:800

Menon KPV (1938) Report on the scheme of investigations on the diseases of coconuts in South India, 1937. Government of India Press, New Delhi, p 16

Menon KPV, Nair UK (1948) The leaf rot disease of coconut in Travancore and Cochin. Indian Cocon J 1:33–39

Menon KPV, Nair UK (1951) Scheme for the investigation of the root and leaf diseases of the coconut palm in South India. Consolidated final report of the work done from 8th March 1937 to 31st March, 1948. Indian Cocon J 5(1):3–19

Mora-Aguilera G (2002) Dispersal potential of lethal yellowing of the coconut palm: a quarantine disease in Mexico. In: Proceedings of expert consultation on sustainable coconut production through control of lethal yellowing disease, Kingston, Jamaica. CFC/FAO/CIB, pp 128–133

Mpunami A, Jones P, Dickinson M, Tymon A, Harrison NA (1997) Detection of coconut lethal disease in Tanzania by use of DNA probes and PCR. In: Eden Green SJ, Ofori F (eds)

International workshop on lethal yellowing-like disease of coconut, Elmina, Ghana, November 1995. Natural Resources Institute, Chatham, pp 205–218
Mpunami AA, Tymon A, Jones P, Dickinson MJ (1999) Genetic diversity in the coconut lethal yellowing disease phytoplasmas of East Africa. Plant Pathol 48:109–114
Mpunami A, Tymon A, Jones P, Dickinson MJ (2000) Identification of potential vectors of the coconut lethal disease phytoplasma. Plant Pathol 49:355–361
Muralidharan A, Jayasanker NP, Antony KJ, Rethinam P (1991) Management of coconut root (wilt) disease. In: Nair MK et al (eds) Coconut root (wilt) disease, CPCRI monograph series no.3. CPCRI, New Delhi, pp 73–80
Myrie W (2002) Current outbreaks of lethal yellowing in Jamaica and the use of molecular diagnostic techniques in phytoplasma detection. In: Proceedings of expert consultation on sustainable coconut production through control of lethal yellowing disease, Kingston, Jamaica. CFC/FAO/CIB, pp 107–115
Myrie W, Paulraj L, Dollet M, Wray D, Been B, McLaughlin W (2006) First report of lethal yellowing disease of coconut palms caused by phytoplasma on Nevis Island. Plant Dis 90:834–834
Myrie W, Oropeza C, Saenz L, Harrison NA, Mercedes Roca M, Cordova I (2011) Reliable improved molecular detection of coconut lethal yellowing phytoplasma and reduction of associated disease through field management strategies. Bull Insectol 64:S203–S204
Myrie W, Douglas CJ, Harrison NA, McLaughlin WA, James M (2012) First report of lethal yellowing disease associated with subgroup 16SrIV,a phytoplasma on St. Kitts in the Lesser Antilles. New Dis Rep 26:25
Nagaraj AN, Davis TA, Menon KPV (1954) Sap transfusion, a new device for virus transmission trials in palms. Indian Cocon J 7:91–98
Nainanayake AD, Wijesekara HTR, Gunathilake I (2013) Disease spreading pattern – GIS mapping. In: Gunasena HPM, Gunathilake HAI, Fernando LCP, Everard IMDT, Appuhamy PAHN (eds) Coconut Research Institute, Sri Lanka, pp 48–52
Nainanayake AD, Gunathilake J, Kumarathungai MDP, Gunawardena PM, Wijesekara HTR (2016a) Limitation in the use of spectral analysis to detect Weligama Coconut Leaf Wilt Disease affected palms in Southern Sri Lanka. COCOS 22:13–24
Nainanayake AD, Kumarathungai MDP, de Silva PHPR (2016b) A survey of land for Weligama Coconut Leaf Wilt Disease affected palms outside the declared boundary in the Southern Province. COCOS 22:57–64
Nambiar PTN, Pillai NG (1985) A simplified method for indexing root (wilt) affected coconut palm. J Plantn Crops 13(1):35–37
Nambiar MC, Prasannakumari TO (1964) Effect of root (wilt) disease on microsporogenesis in coconut (*Cocos nucifera* L.). Indian Cocon J 17:93–100
Narasimhachari CL, Prasadji JK, Rajamannar M (1991) Survey and confirmation of Tatipaka diseased palms in central delta of river Godavari in Andhra Pradesh. Indian Cocon J 22(3):7–10
Nejat N, Sijam K, Abdullah SNA, Vadamalai G, Dickinson M (2009a) Molecular characterization of a phytoplasma associated with Coconut Yellow Decline (CYD) in Malaysia. American J Appl Sci 6:1331
Nejat N, Sijam K, Abdullah S, Vadamalai G, Dickinson M (2009b) Phytoplasmas associated with disease of coconut in Malaysia: phylogenetic groups and host plant species. Plant Pathol 58:1152–1160
Nejat N, Sijam K, Abdullah SNA, Vadamalai G, Sidek Z, Dickinson M, Edizioni ETS (2010) Development of a TaqMan real-time PCR for sensitive detection of the novel phytoplasma associated with coconut yellow decline in Malaysia. J Plant Pathol 92(3):769–773
Nejat N, Cahill DM, Vadamalai G, Ziemann M, Rookes J, Naderali N (2015) Transcriptomics-based analysis using RNA-Seq of the coconut (*Cocos nucifera*) leaf in response to yellow decline phytoplasma infection. Springer, Berlin/Heidelberg. Mol Gene Genomics 290(5):1899–1910
Nienhaus F, Steiner KG (1976) Mycoplasma like organisms associated with Kaincope disease of coconut palms in Togo. Plant Dis Rep 60:1000–1002

Nienhaus F, Schuiling M, Gliem G, Schinzer U, Spittel A (1982) Investigations on the etiology of the lethal disease of coconut palm in Tanzania. Z Pjlkrankh Pjlschutz 89:185–193

Nipah JO, Jones P, Dickinson MJ (2007) Detection of lethal yellowing phytoplasma in embryos from coconut palms infected with Cape St. Paul wilt disease in Ghana. Plant Pathol 56:777–784

Nkansah JP, Philippe R, Quaicoe RN, Dery SK, Ransford A (2009) Cape Saint Paul Wilt Disease of coconut in Ghana: surveillance and management of disease spread. Oléagineux 16:111–115

Ntushelo K, Elliott ML, Harrison NA (2013) Palm yellows phytoplasmas and their genetic classification. African J Biotech 12:3376–3382

Nutman FJ, Roberts PM (1955) Lethal Yellowing: the unknown disease of coconut palm in Jamaica. Emp J Exp Agric 23:257–267

Odewale JO, Nair RV, Enaberue L (2006) Field resistance to lethal yellowing disease in some coconut varieties in Nigeria. Trop Agric 83(4):135

Odewale JO, Odionwaya G, Osagie JI, Ahanon JM (2010) Rate of lethal yellowing disease (LYD) spread in coconut (*Cocos nucifera* L) plantation of tall inter planted with Dwarf varieties. In: Proceedings of the 19th conference of Botanical Society of Nigeria, Umaru Musa Yar"addua University, Katsina, Nigeria, p 47

Ofori F, Nkansah-Poku J (1997) Cape St Paul Wilt disease of coconut in Ghana: history of its occurrence and spread. In: Proceedings of international workshop on lethal yellowing-like diseases of coconut. Natural Resources Institute, Chatham, pp 27–32

Oropeza C, Cordova I, Chumba A, Narváez M, Sáenz L, Ashburner R, Harrison N (2011) Phytoplasma distribution in coconut palms affected by lethal yellowing disease. Ann Appl Biol 159:109–117

Oropeza C, Cordova I, Puch-Hau C, Castillo R, Chan JL, Sáenz L (2017) Detection of lethal yellowing phytoplasma in coconut plantlets obtained through *in vitro* germination of zygotic embryos from the seeds of infected palms. Ann Appl Biol 171:28–36

Osagie IJ, Ojomo EE, Pilet F (2013) An update on Awka wilt disease of coconut caused by phytoplasma in Nigeria. Acta Phytopathologica Sinica 43(suppl):204

Osagie IJ, Ojomo EE, Pilet F (2016) Detection of phytoplasma associated with Awka wilt disease of coconut palms in Nigeria by polymerase chain reaction. Intl J Appl Res Tech 5(4):49–55

Pandit SV, Simhachalam G, Lal SB (1969) A note on Tatipaka or decline disease of coconut in Andhra Pradesh. India. In: Proceedings of 3rd session. FAO Technical Working Party on Coconut Production, Protection and Processing, Jakarta, 9–19 September 1968. Paper No.41, p 182

Parthasarathy MV (1974) Mycoplasma-like organisms associated with lethal yellowing disease of palms. Phytopathol 64:667–674

Perera L, Meegahakumbura MK, Wijesekara HTR, Kalani NGA, Munasing CE, Fernando WBS, Dickinson MJ (2010) Detection of Weligama coconut leaf wilt disease in Sri Lanka by polymerase chain reaction. In: George VT, Krishnakumar V, Augustine JB, Niral V, Josephrajkumar A (eds) Proceedings of the international conference on coconut biodiversity for prosperity. Niseema Printers and Publishers, Kochi, p 146

Perera L, Meegahakumbura MK, Wijesekara HRT, Fernando WBS, Dickinson M (2012) A phytoplasma is associated with Weligama Coconut Leaf Wilt Disease in Sri Lanka. J Plant Pathol 94:205–209

Perera SACN, Herath HMNB, Wijesekera HTR, Subhathma WGR, Weerakkody WATL (2015) Evaluation of coconut germplasm in Weligama and Matara area of the Southern Province of Sri Lanka for resistance to Weligama Coconut Leaf Wilt Disease. COCOS 21:15–20

Philippe R, Reignard S, Descamps S, Nkansah-Poku J, Quaicoe R, Pilet F, Fabre S, Dollet M (2009) Study on the transmission of coconut Lethal Yellowing in Ghana. Oléagineux 16:102–106

Pillai NK (1911) Naleekeram. Vidyabhivardhini Press, Quilon, p 112

Pillai NG, Raju CA (1985) A simple pressure injection device for application of antibiotics on coconut palm. J Plantn Crops 13:151–154

Pillai NG, Lal SB, Shanta P (1973) Distribution and intensity of root (wilt) disease of coconut in Kerala. J Plantn Crops 1(Suppl):107–112

Pillai NG, Sasikala M, Mathen K (1980) Epidemiology of coconut root(wilt) disease. J Plantn Crops 8:90–97

Pillai NG, Chowdappa P, Solomon JI, Mathew J (1991) Remission of symptoms of root (wilt) disease of coconut injected with Oxytetracycline-HCI. J Plantn Crops 19(1):14–20

Pilotti CA, Dewhurst CF, Liefting LW, Kuniata L, Kakul T (2014) Putative vectors of a phytoplasma associated with coconut (*Cocos nucifera*) in Madang Province Papua New Guinea. Intl J Agric For 4:365–372

Plavsic-Banjac B, Hunt P, Maramorosch K (1972) Mycoplasma like bodies associated with lethal yellowing disease of coconut palms. Phytopathol 62:298–299

Quaicoe RN, Dery SK, Philippe R, Baudouin L, Nipah JO, Nkansah-Poku J et al (2009) Resistance screening trials on coconut varieties to Cape Saint Paul Wilt Disease in Ghana. Oléagineux 16:132–136

Quillec G, Morin JP, Renard JL, Marian D (1978) Disease of young coconut palms. Oleagineux 33:500–501

Quiroz L (2002) Information on coconut Lethal Yellowing in Belize. In: Proceedings of expert consultation on sustainable coconut production through control of Lethal Yellowing Disease. CFC/FAO/CIB, Kingston, p 87

Radha K, Lal SB (1972) Diagnostic symptoms of root (wilt) disease of coconut. Indian J Agric Sci 42:410–413

Rajagopal V, Patil KD, Mathew C, Amma BSK (1986a) Abnormal stomatal opening in coconut palms affected with root (wilt) disease. J Exp Bot 37:1398–1405

Rajagopal V, Patil KD, Mathew C, Amma BSK, Varkey T (1986b) Water relation studies in root (wilt) affected coconut palms. In: Final report Central Plantation Crops Research Institute, Regional Station, Kayamgulam, Kerala, India, p 24

Rajagopal V, Mathew C, Varkey T, Joseph KV (1998) Physiology and biochemistry. In: Nampoothiri KUK, Koshy PK (eds) Coconut root (wilt) disease. Central Plantation Crops Research Institute, Kasaragod, pp 65–73

Rajamannar M, Rao AP, Ramapandu S, Subbiah J (1984) Etiology and control of Tatipaka disease of coconut – an appraisal. In: Proceedings of all India symposium coconut diseases. Coconut Research Station, Veppankulam, pp 35–39

Rajamannar M, Prasadji JK, Rethinam P (1993) Tatipaka disease of coconut. Current status. In: Nair MK, Khan HH, Gopalasundaram P, Rao EVVB (eds) Advances in coconut research and development. Oxford and IBH Publishing Co. Pvt Ltd, New Delhi, pp 591–595

Rajamannar M, Prasadji JK, Rethinam P (1994) Tatipaka disease of coconut. In: Chadha KL, Rethinam P (eds) Advances in horticulture 10 – plantation and spice crops, Part 2. Malhotra Publishing House, New Delhi

Rajan P (2011) Transmission of coconut root (wilt) disease through plant hopper, *Proutista moesta* Westwood (Homoptera: Derbidae). Pest Manag Hort Ecosyst 17:1–5

Rajan P, Mathen K (1985) *Proutista moesta* (Westwood) and other additions to the insect fauna on coconut palms. J Plantn Crops 13:135–136

Rajan P, Nair CPR, Solomon JJ, Geetha L (2002) Identification of Phytoplasma in the salivary glands of *Proutista moesta* (West wood), a putative vector of coconut root (wilt) disease. J Plantn Crops 30(2):55–57

Ramadasan A, Shanta P, Lal SB (1971) Resistance or susceptibility, age of bearing and yield in young coconut palms in relation to the development of root (wilt) disease. Indian J Agric Sci 41:1107–1109

Ramapandu S, Rajamannar M (1981) Further investigations on the etiology and control of Tatipaka disease of coconut. Paper presented at the third international symposium of plant pathology, New Delhi

Ramaswamy M, Soumya VP, Nair S, Rani RG, Rao GP (2016) PCR detection of phytoplasmas from different tissues of root (wilt) diseased coconut palms. Phytopathogenic Mollicutes 6(1):23–28

Randles JW, Hatta T (1980) Etiological studies on virus like diseases of coconut and cardamom in India. In: Biennial report of the Waite Agric Res Inst 1978–1979, Melbourne, Australia

Rao AP (1966) Identification of Tatipaka disease of Coconut. Andhra Agric J 13:112–113

Rao PG, Reddy GS, Subbiah J (1956) A new disease of coconut in Andhra State. Indian Cocon J 9:215–222

Rethinam P, Rajamannar M, Narasimhachari CL (1989) Tatipaka disease of coconut in Andhra Pradesh. Indian Cocon J 20(1):1–4

Rhode W, Kullaya A, Mpunami A, Becker D (1993) Rapid and sensitive diagnosis of mycoplasma-like organisms associated with lethal disease of coconut palm by a specifically primed Polymerase chain reaction for the amplification of 16 SrDNA. Oleagineux 48:319–322

Romney DH (1972) Past studies on and present status of lethal yellowing disease of coconuts. Intl J Pest Manag 18:386–395

Romney DH (1983) Brief review of coconut lethal yellowing. Indian Cocon J 13:1–8

Romney DH, Harries HC (1978) Distribution and impact of lethal yellowing in the Caribbean. In: Proceedings of the 3rd meeting of the international council of lethal yellowing, University of Florida Agricultural Research Centre, Fort Lauderdale, 78:6–7

Russel WC, Newman C, Williamson DH (1975) A simple cytochemical technique for demonstration of DNA in cells infected with mycoplasmas and viruses. Nature 25:461–462

Sahasranaman KN, Pillai NG, Jayasankar NP, Potti VP, Varkey T, Kamalakshy Amma PG, Radha K (1983) Mixed farming in coconut gardens: economics and its effect on root (wilt) disease. In: Nayar NM (ed) Coconut research and development. Wiley Eastern, New Delhi, pp 160–165

Sasikala M, Mathen K, Govindankutty MP, Solomon JJ, Geetha L (1988) Transmission of a mycoplasma-like organism from *Cocos nucifera* with root(wilt) disease to *Catharanthus roseus* by *Cassytha filiformis*. Netherlands J Plant Pathol 94:191–194

Sasikala M, Amma BSK, Rajagopal V (1998) Diagnosis. In: Nampoothiri KUK, Koshy PK (eds) Coconut root (wilt) disease. CPCRI, Kasaragod, pp 45–51

Sasikala M, Chithra R, Solomon JJ, Rajeev G (2001) Development of DAC – indirect ELISA for the rapid detection of coconut root (wilt) disease. CORD 17:23–35

Sasikala M, Prakash VR, Ajithkumar R, Chandra Mohanan R (2004) Selection of root (wilt) disease free coconut elite mother palms using serological test for production of quality planting materials for disease-prevalent tracts. Indian Cocon J 35:16–19

Sasikala M, Rajeev G, Prakash VR, Amith S (2010) Modified protocol of ELISA for rapid detection of coconut root (wilt) disease. Central Plantation Crops Research Institute (CPCRI), Kasaragod, India. J Plantn Crops 38(1):16–19

Schneider B, Ahrens U, Kirkpatrick BC, Seemuller E (1993) Classification of plant-pathogenic mycoplasma- like organisms using restriction-site analysis of PCR- amplified 16s rDNA. J Gen Microbiol 139:519–527

Schuiling M, Kaiza DA, Mpunami A (1992a) Lethal disease of coconut palm in Tanzania 2-History, distribution, and epidemiology. Oleagineux 47:516–521

Schuiling M, Mpunami A, Kaiza DA, Harries HC (1992b) Lethal disease of coconut palm in Tanzania 3- Low resistance of imported germplasm. Oleagineux 47:693–697

Sears BB, Kirkpatrick BC (1994) Unveiling the evolutionary relationships of plant pathogenic mycoplasma like organisms. ASM News 60:307–312

Seymour CP, Miller JW, Roberts DA (1972) An outbreak of lethal yellowing of coconut palms in Miami, Florida. Phytopathol 62:788

Shanta P, Menon KPV (1959) The virus nature of wilt (root) disease of coconut – experimental transmission of it into cowpea (*Vigna sinensis* Endl.) In: Proceedings of conference, Coconut Research Workers India, pp 268–271

Sharples A (1928) Palm diseases in Malaya. Malay Agric J 16(9–10):313–360

Siriwardhana PHAP, Gunawardena BWA, Steve Millington SJ (2012) Detection of phytoplasma associated with Waligama Coconut Leaf Wilt Disease in Sri Lanka by loop mediated isothermal amplification assay performing alkaline polyethylene glycol based DNA extraction. Microbiol Biotech Res 2(5):712–716

Sitepu D, Mangindaan H, Mawikere J (1988) Outbreak of a coconut wilt disease in Central Kalimantan. In: UNDP/FAO integrated coconut pest control project, annual report 1988. Balai Penelitian Kelapa, Manado, North Sulawesi
Solomon JJ, Govindankutty MP, Nienhaus F (1983) Association of mycoplasma-like organisms with the coconut root (wilt) disease in India. Z Pjlkrankh Pjlschutz 90:295–297
Solomon JJ, Govindankutty MP, Mathen K (1987) Detection of mycoplasma like organism in root (wilt) disease affected coconut and its putative vector. In: Regional workshop on plant mycoplasma, New Delhi, India
Solomon JJ, Nair CPR, Srinivasan N, Gunasekaran M, Sasikala M (1999) Coconut root (wilt) – the malady and remedy. J Plantn Crops 27(2):71–92
Sosamma VK, Koshy PK (1991) Etiology-nematodes. In: Root (wilt) disease, Monograph series no. 3. Central Plantation Crops Research Institute, Kasaragod, pp 19–26
Srinivasan N (1991) Occurrence of coconut leaf rot in relation to root (wilt) disease. Indian Cocon J 21(10):14–18
Srinivasulu B, Nagamalleswari DY, Kumar KVK, Aruna K, Prasadji JK, Rao DVR (2006) Transmission electron microscopy confirms phytoplasmal etiology of Tatipaka disease of coconut. J Plantn Crops 34(3):500–501
Steiner KG (1976a) Epidemiology of Kainkope disease of coconut palms in Togo. Plant Dis Rep 60(7):613–617
Steiner KG (1976b) Remission of symptoms following Tetracycline treatment of coconut palms affected with Kaincope disease. Plant Dis Rep 60:617–620
Subbiah J, Rao PG (1963) Studies in coconut decline in Andhra. Andhra AgriC J 10:58–67
Summanwar AS, Raychaudhuri SP, Jagadish Chandra K, Namaprakash LSB (1969) Virus associated with coconut root (wilt) disease. Curr Sci 38:208–210
Tanne E, Boudon-Padieu E, Clair D, Davidovich M, Melamed S, Klein M (2001) Detection of phytoplasma by polymerase chain reaction of insect feeding medium and its use in determining vectoring ability. Phytopathol 91:741–746
Tsai JH (1980) Lethal yellowing of coconut palms – search for a vector. In: Harris K, Maramorosch K (eds) Vectors of plant pathogens. Academic, New York, pp 177–200
Tymon A, Jones P (1997) Comparative analysis of coconut phytoplasmas from East and West Africa. In: Eden-Green SJ, Ofori F (eds) International workshop on lethal yellowing-like disease of coconut, Elmina, Ghana, November 1995. Natural Resources Institute, Chatham, pp 197–203
Tymon AM, Jones P, Harrison NA (1998) Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. Ann Appl Biol 132:437–452
Varghese MK (1934) Diseases of coconut palm, p 105
Varkey T, Davis TA (1960) Studies on coconut pollen with reference to the leaf and root (wilt) diseases. Indian Cocon J 14:1–7
Vázquez-Euán R, Harrison N, Narvaez M, Oropeza C (2011) Occurrence of a 16SrIV group phytoplasma not previously associated with palm species in Yucatan, Mexico. Plant Dis 95(3):256–262
Waidyarathne KP, Samarasinghe S (2014) Artificial neural networks to identify naturally existing disease severity status. Neural Comput Appl. https://doi.org/10.1007/s00521-014-1572-6
Warokka JS (1998) The impact and etiology of coconut wilt disease in Indonesia. In: Allorerung D, Harries HC, Jones P, Warokka JS (eds) Proceedings of the workshop on lethal diseases of coconut caused by phytoplasma and their importance in Southeast Asia. APCC, Jakarta, pp 27–35
Warokka JS, Jones P, Dickinson MJ (2006) Detection of phytoplasmas associated with Kalimantan wilt disease of coconut by the polymerase chain reaction. J Littri 12:154–160
Weerakkody WATL (2010) Impact of Weligama Coconut Leaf Wilt Disease (WCLWD) on morphological, physiological and yield aspects of affected coconut palms. A Project Report presented to the University of Peradeniya, Sri Lanka. dlib.pdn.lk/bitstream123456789/3147/Weerakody%202010.pdf

Weerakoon WMSSB, Yalegama LLWC, Fernandopulle MND (2011) Evaluation of copra quality and quantity of Weligama Wilt affected coconuts. In: Proceedings of 11th Agri Res Symp, pp 319–322

Wei W, Davis RE, Lee I-M, Zhao Y (2007) Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. Intl J Syst Evol Microbiol 57:1855–1867

Wijesekara HTR, Perera L, Wickramananda IW, Herath I, Meegahakumbura MK, Fernando WBS, De Silva PHPR (2008) Preliminary investigation on Weligama coconut leaf Wilt disease: a new disease in Southern Sri Lanka. In: Nainanayake NPAD, Everard JMDT (eds) Proceedings of the second symposium on plantation crop research – Export competitiveness through quality improvements. Coconut Research Institute, Lunuwila

Wijesekara HTR, Perera AAFLK, Meegehekumbura MGMK, Dassanayaka EM, Ranasinghe C (2013) Serological and Molecular Techniques. In: Gunasena HPM, Gunathilaka HAJ, Fernando LCP, Everard JMDT, Appuhamy PAHN (eds) Weligama Coconut Leaf wilt disease six years after. Coconut Research Institute of Sri Lanka, Lunuwila, pp 56–70

Yankey EN, Swarbrick PJ, Nipah JO, Quaicoe RN, Dickinson MJ, Edizioni ETS (2014) Detection of the Cape St. Paul wilt phytoplasma in coconut palms in Ghana through the combination of visual symptoms assessment and molecular diagnosis using a secA gene based assay. J Plant Pathol 96(2):281–285

Zizumbo-Villarreal DP, Colunga-García Marín M, Fernández-Barrera N, Torres-Hernández, Oropeza C (2008) Mortality of Mexican coconut germplasm due to lethal yellowing. PGR Newsletter no, vol 156, pp 23–33

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