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REVIEW ARTICLE

COCONUT ROOT WILT - THE MALADY AND REMEDY

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INTRODUCTION

Coconut root wilt, an endemic disease in Kerala, India, is of concern to the coconut growers because of the quantum of economic loss incurred and the non-availability of an immediate control measure. Recent studies have, however, contributed to the understanding of the etiology, transmission and management of the disease. This review aims to update the available information on this disease and to identify future directions of research.

How serious/important is the disease?

Root wilt disease (RWD) was first noticed after the great floods of 1882 in three isolated pockets 50 km away from each other in the erstwhile princely State of Travancore, presently included in the Kerala State (Butler, 1908; Pillai, 1911; Varghese, 1934). Since then it has spread in all directions from the original foci of infection and according to a survey of 1984-85 the disease occurred in a contiguous manner in 0.41 million ha in eight southern districts and in isolated pockets in the northern districts of Kerala, and also in districts of Tamil Nadu bordering Kerala State. The intensity of the disease ranged from 1.5% in Trivandrum district to 75.6% in Kottayam district. Data on the extent of disease incidence in the eight districts was updated in 1996 by a sample survey (Dept. of Agriculture, Govt. of Kerala, 1997). The incidence ranged from 2.1% in Trivandrum district to 48.0% in Alleppey district (Table 1). Overall reduction in disease incidence since 1984, from 32.37 to 24.05% is attributed to the removal of about 59 lakh diseased palms, replanting with quality seedlings, adoption of disease management practices and crop conversion from coconut to rubber. The annual loss due to the disease was estimated to be around 968 million nuts (CPCRI, 1985). The total estimated monetary loss in terms of loss in husk, copra yield, leaf number and quality of leaves on the basis of 1984 price index was of the order of about Rs. 3000 million.

Table 1: ***Disease percentage***

Districts	Survey 1984/85	Survey 1996/97
1. Trichur	2.60	6.19
2. Ernakulam	34.52	33.01
3. Idukki	34.18	33.56
4. Kottayam	75.63	36.50
5. Alleppey	70.59	48.03
6. Pathanamthitta	38.22	37.80
7. Quilon	28.55	25.97
8. Trivandrum	1.52	2.09
Overall	32.37	24.05

RWD is non-lethal but debilitating and palms of all age groups are affected. In palms contracting the disease in the seedling stage, delayed flowering and reduction in yield were observed (Ramadasan *et al,* 1971).

Can the disease be easily identified?

The diagnostic symptom of the disease is the characteristic bending or ribbing of the leaflets termed 'flaccidity'. Foliar yellowing and marginal necrosis are the other associated symptoms mostly observed in the older leaves (Radha and Lal, 1972). In juvenile palms and seedlings, flaccidity is often the only symptom and yellowing is virtually absent (Radha and La, 1972). Softening and whitening of leaflets of spindle leaves and interveinal yellowing in the leaves of middle whorl of RWD affected palms were described as important symptoms for early diagnosis of the disease (Dwivedi *et al,* 1979). Instances of diseased palms having their outer leaves quite healthy but with yellowing of leaves in some of the inner whorls was noticed earlier also (Menon, 1937) This has a resemblance to "flagging" observed in Lethal Yellowing affected palms in the Caribbean (Mc Coy, 1983). The leaves produced after the contraction of the disease are stunted and are shed quickly, thus producing a steady reduction in size of the crown (Menon, 1937).

Drying up of spathe and necrosis of spikelets extending from tip towards the base was observed in unopened inflorescences in certain cases (Varghese, 1934; Maramorosch, 1964). However, this is not a consistent symptom as observed in the lethal yellowing disease in Florida and the Caribbean. A high percentage of pollen produced was either sterile or with low viability (Varkey and Davis, 1960). Meiotic irregularities were also observed (Nambiar and Prasannakumari, 1964).

Shedding of immature nuts is yet another symptom observed at times in bearing palms. (Menon, 1937). Such "shedding of buttons" is a characteristic symptom in lethal yellowing disease in the Caribbean and African countries (Mc Coy, 1983; Steiner, 1976). Reduction in yield of nuts upto 80% was noticed in palms in the advanced stages of the disease (Radha *et al,* 1962). The nuts from diseased palms have thinner husk and fibres are weaker and less firm (Varghese, 1934). The kernel is thinner and never dries up into hard brittle copra externally. However, the meat remains soft and flexible. Although no significant changes in the biochemical constituents of the coconut meat due to RWD were observed, the quality of coconut water was found to be affected (Sosamma Cherian and Chandrasekharan Nair, 1993). The loss of copra/oil per nut is 9.0/11.3 percent (CPCRI, 1985) and the oil also looses its flavour (Menon, 1937).

Extensive rotting of root system (Menon and Pandalai, 1958) concomitant with the reduction in capacity of palms to regenerate new roots with the progress of the disease (Michael, 1964) affected the vigour and contributed to its rapid decline.

An indexing method for quantifying the disease intensity has been developed giving due weightage and grade points to the three major foliar symptoms - flaccidity, yellowing and necrosis. The formula thus developed helps in assessing the intensity of the disease precisely (George and Radha, 1973; Nambiar and Pillai, 1985).

What is leaf rot? How is it related to RWD?

Leaf rot disease (LRD) is a disease of fungal complex and occurs superimposed on more than 65% of RWD affected palms (Srinivasan, 1991). A clear correlation exists between LRD and RWD. LRD appears especially on the spindles, as minute water soaked lesions. These lesions enlarge, coalesce freely leading to extensive rotting of tissues under the favourable weather conditions of high rainfall and high relative humidity (Menon and Nair, 1951). As the leaves mature, the progress of infection is retarded. Thus the emerging spindle leaves play a critical role in the LRD incidence and perpetuation. Successive leaves may contract the disease and rotting progresses to varying degrees giving a fan-like appearance to the leaves and resulting in linear disease progression (Srinivasan and Gunasekaran, 1992).

Colletotrichum gloeosporioides (Penzig) Penzig & Sacc. and *Exserohilum rostratum* (Drechsler) Leonard & Suggs. are the main pathogens of LRD (Srinivasan and Gunasekaran, 1993, 1994, 1996a). *C. gloeosporioides* is the pathogen most commonly associated with the disease during monsoon seasons. Hence, it is implicated as the principal pathogen involved in the initiation of LRD during that period (Srinivasan and Gunasekaran, 1996b). RWD palms become susceptible to LRD pathogens and this brings about steady deterioration of the affected palms.

Can RWD be diagnosed at an early stage?

Diseased palms are generally identified based on foliar symptoms. However, this could be misleading as palms growing in shaded condition exhibit etiolation and bending of leaflets simulating flaccidity. Similarly, yellowing due to Mg deficiency is also known (Lal, 1968). Hence, reliable diagnostic tests for the early detection of the disease is important. A sero-diagnostic test (Solomon *et al.* 1983a) and a physiological test based on stomatal resistance (Rajagopal *et al.* 1986) which could detect the disease status 6 to 24 months before the manifestation of foliar symptoms, have been standardized.

Does soil type has any relation to RWD?

The disease is prevalent in all the major soil types of Kerala State. However, the spread is faster in sandy, sandy loam, alluvial and clayey soils than in laterite. The rate of spread of the disease was found to be 1 to 4 km/year from the nearest source of inoculum (Pillai, 1981). Relatively higher disease incidence is noticed in water-logged low lying areas adjacent to rivers and canals and in kari soils (Pillai *et al.* 1973). The spread of the disease is not contiguous but erratic and in jumps. Such spread in leaps is also reported in other coconut diseases of similar etiology *viz*, the Caribbean lethal yellowing disease (Mc Coy, 1983) and Cape St. Paul wilt disease in Ghana (Derry and Philippe, 1995). The pattern of spread characterized by frequent jumps or leaps is suggestive of the possible involvement of an aerial vector (Pillai *et al*, 1980). Healthy pockets surrounded by diseased gardens and individual healthy palms in the midst of diseased palms without any spread to neighbouring palms for several years is also noticed (Menon, 1943).

What is the cause of RWD?

The sporadic occurrence, spreading and infectious nature of the disease

implied the involvement of a pathogen as the cause of the disease. Concurrently, the involvement of physiological, soil and nutritional factors in the etiology of the disease was also investigated.

Is fungus the cause?

Bourdillon in 1906 first reported that the disease was 'fungoid' in nature (Varghese, 1934). Butler (1908) surmised that the roots of diseased palms are rotten by the attack of a parasitic fungus suspected to be a species of *Botryodiplodia* and that probably the root-rot caused by it is sufficient to produce the disease. Further isolations made since then led to the identification of *Rhizoctonia solani* Lutu, and *R. bataticola* (Taub.) Butl. besides *Botryodiplodia theobromae* Pat. (Menon and Nair, 1949) from the rotting root system of diseased palms. Pathogenicity experi- ments with *R. solani* and *R. bataticola* in the field as well as under pot culture conditions although induced rotting of roots, did not produce the diagnostic foliar symptoms (Menon and Nair, 1951). In later studies *Cylindrocarpon effusum* Bugn. and *Fusarium equiseti* (Corda) Sacc. were isolated from apparently healthy roots of diseased palms (Joseph, 1978). *C. effusum* and *C. lucidum* were also isolated from the burrowing nematode lesions on the roots (Sosamma and Koshy, 1978). However, pathogenicity experiments with *C. effusum* and *F. equiseti* singly and in combination on coconut seedlings grown in individual field tanks also did not produce the symptoms of RWD (Joseph and Lily, 1991).

Can bacteria be the cause?

Menon and Nair (1951) reported the isolation of two bacteria from the roots of RWD affected palms but no causative role was ascribed to any one of them. Srivastava *et al,* (1969) observed characteristic streaming movement of bacteria in the vascular tissues of diseased palms and isolated a bacterium tentatively identified as *Pseudomonas* sp. However, its pathogenicity was not established. In an enriched medium containing 15% (w/v) coconut root extract solidified with agar, George *et al,* (1976) isolated an off-white bacterium identified as *Enterobacter cloacae* (Jordan) Hornaeche and Edwards from the stelar portions of freshly collected root tips of diseased palms. Inoculation of coconut seedlings with *E. cloacae* also did not produce the symptoms of the disease, thereby ruling out the involvement of bacteria in the disease incidence (Jayasankar and George, 1991).

What is the role of nematodes?

The nematological investigations on the disease were prompted by certain indications on the soil-borne nature of the disease which implied the involvement of either a soil-borne pathogen or a pathogen transmitted through 'soil-borne' vector. Examination of soil and roots collected from disease prevalent and disease-free tracts revealed the presence of 39 genera of plant parasitic nematodes including species of *Xiphinema, Longidorus* and *Trichodorus* which are known vectors of virus diseases and the burrowing nematode *Radopholus similis* (Weischer, 1967; Mathen, 1969; Mathen *et al,* 1970; Khan *et al,* 1971; Koshy *et al,* 1979). The results of initial investigation on the high population of *R. similis* in RWD affected as well as healthy palms in the diseased tract and the notoriety of this nematode as having caused great havoc in citrus and banana in other countries prompted assessing its role in the etiology of the disease.

In a pathogenicity experiment coconut seedlings inoculated with upto one million *R. similis* did not reproduce the RWD symptoms even after eight years of experimentation (Sosamma and Koshy, 1991). Extensive surveys covering RWD-endemic and RWD-free areas also could not bring out a correlation between nematode infestation and incidence of the disease (Koshy *et al,* 1978). In this context, the observation of Weischer (1967) although based on results of limited number of samples, is of significance. Weischer (1967) concluded then itself that the low population density of nematodes and wide occurrence and the general distribution pattern of the disease indicate that plant parasitic nematodes can be excluded as the primary cause of RWD.

Pathogenicity trials conducted on coconut seedlings using the biological agents viz., the fungi (*F. equiseti, C. effusum),* the burrowing nematode (*R. similis*) and the bacterium *(E. cloacae)* singly and in combination did not induce the RWD symptoms implying that these organisms do not have any role in RWD (Joseph and Lily, 1991; Jayasankar and George, 1991; Sosamma and Koshy, 1991).

Is virus the cause?

Based on the systemic nature and resemblance of symptoms to other known plant virus diseases, a viral etiology was proposed (Nagaraj *et al,* 1954). This theory gained further impetus with the claims of positive transmission of the disease to coconut by sap inoculation and through the insect vector *Stephanitis typica* under field condition (Nagaraj and Menon, 1956) and insect-proof condition (Shanta *et al,* 1964).

Cowpea was considered as an indicator plant for the presumed 'virus' (Shanta and Menon, 1960) and the physical properties of the 'virus' were worked out using cowpea as a test plant (Shanta and Menon, 1961). However, the symptoms on cowpea were found to be very inconsistent in subsequent studies and after detailed investigation it was concluded that it cannot be used as a reliable indicator plant (Sasikala and Pillai, 1978). The peculiar nature of symptoms on cowpea and lack of proof of its passage through bacterial filters prompted Holmes (1965) to surmise that although the pathogen resembled a virus, it might as well be a related organism like a spirochaete or sporozoa.

Summanwar *et al,* (1969) reported isolation of a strain of Tobacco Mosaic Virus (TMV) from roots and leaves of diseased coconut palms. However, detailed electron microscopic and pathogenicity studies (Shanta *et al,* 1975) and serological studies have ruled out the association of TMV with the RWD. Presence of icosahedral particles of 56 nm diam. in epidermis and ground parenchyma cells of diseased palms (Maramorosch and Kondo, 1977) was reported. These particles were later identified as nothing but plasmodesmata sectioned in tangential plane (Parthasarathy, 1978). Polyacrylamide gel electrophoretic analysis of isolated nucleic acid from diseased palm did not indicate the association of viroid either (Randles and Hatta, 1980). Hence involvement of virus and viroid with RWD is also ruled out.

Can it be a physiological disorder?

Earlier investigations attributed the disease to certain physiological factors. The physiological changes

observed in diseased palms were derangement in absorption, uptake and transport of water through the trunk (Davis, 1964; Ramadasan, 1964), permeability changes in leaf and root tissues resulting in leaching of metabolites (CPCRI, 1977), impairment of stomatal mechanism (Rajagopal *et al,* 1986), higher respiration rate (Michael, 1978), derangement in translocation and distribution of sugars (Mathew, 1977), altered nitrogen metabolism (Varkey *et al,* 1969), accelerated phenol metabolism with a fall in phenol content and increase in phenol oxidising and synthesising enzymes (Mathew *et al,* 1991). Such malfunctions encountered in diseased palms points to the involvement of a pathogen-mediated altered host metabolism than of a physiological disorder.

Can nutritional deficiency or soil toxicity induce the disease?

Soil sickness characterised by low pH, inadequate drainage, poor aeration, low microbial activity and nutrient imbalances were implicated to have a role in the disease (Menon *et al,* 1950, 1952; Pandalai *et al,* 1958 a,b; Verghese, 1961). However, detailed analysis of soil and tissue samples from disease free and disease endemic areas in Kerala has ruled out the possibility of the direct involvement of any major nutrients in the incidence of the disease (Pillai, 1975; Pillai *et al,* 1975). Cecil (1975) observed that the Ca and Mg contents of healthy palm were significantly higher than those of apparently healthy and diseased palms. However, nutritional trials with different levels of major and secondary nutrients, Ca and Mg could neither control the disease nor prevent the fresh incidence of the disease (Cecil, 1981; Cecil *et al,* 1982; Cecil and Amma, 1991). The role of micronutrients in the incidence of the disease has also been ruled out (Davis and Pillai, 1966; Khan *et al,* 1985; CPCRI., 1986).

Valiathan *et al,* (1992) observed a reciprocal relationship between magnesium and cerium as a common geochemical basis for coconut root wilt disease and human cardiomyopathy that warranted further study.

Wahid (1998) opined that the soils of disease-free and diseased tract are geochemically different and the various nutrient rare earth elements (REE) ratios showed significant differences. Further studies are suggested to zero down to a few elements that are likely to be responsible for the incidence of the disease or for its aggravation.

What is the cause of RWD?

Ultra-structural studies of the vascular tissues revealed the presence of phytoplasma, earlier referred as mycoplasma-like organisms (MLOs), in sieve tubes of roots, tender stem, petiole and developing leaf bases of RWD affected palms (Solomon *et al,* 1983b). Phytoplasmas are plant pathogenic mycoplasmas that are non-helical, non-culturable and transmitted by arthropod insect vectors (International Committee on Systematic Bacteriology-Sub committee on the Taxonomy of Mollicutes, 1993).

Pleomorphic forms ranging from circular to oval and occasionally beaded or filamentous ones are also observed. The coccoid forms are in the size range of 250-400 nm, limited by well defined trilamellar unit membrane and contain DNA strands and peripherally dispersed ribosomes (Solomon, 1991). The phloem parenchyma and companion cells are totally devoid of the organism.

Phytoplasms are distributed in an uneven manner in the vascular tissues and are found in increasing numbers in the 'sink' region notably the heart tissues, rachilla of developing inflorescence and root tips. Degenerated or moribund forms are often observed in mature tissues. (Solomon *et al,* 1987). Neither every sieve tube nor all vascular bundles contained phytoplasma. Some of the individual vascular elements were totally free of the organism or contained fewer bodies in parietal position (Solomon, 1997). Such uneven distribution of the organism had been reported in lethal yellowing disease of palms (Parthasarathy, 1974a; Thomas, 1979), X-disease of *Prunus* (Garnet and Gilmer, 1971) and certain other witches broom diseases (Hiruki and Shukla, 1973; Seliskar *et al,* 1973). Phytoplasma concentration in Coconut and *Veitchia merrillii* palms was among the lowest of the 21 palm species studied and five percent of the vascular bundles alone contained the organism in a very low concentration in coconut (Thomas, 1979).

The cell walls of the phytoplasma-harboring cells and the adjoining ones are often thickened, the cytoplasm granulated and contain vesicle-like structures (Solomon *et al,* 1983b). These cells frequently have fibrillar tubules with electron dense granules (Solomon and Geetha, 1993). Dollet and Gianatti (1976) described the fibrils in coconut palms affected by Kaincope disease as P-proteins. Over production of P-proteins is recorded in TMV infected tobacco plants (Esau, 1968). The P-proteins which are similar to microfilaments are supposed to provide the motive force to assimilate movement in sieve elements (Hepler and Palevitz, 1974). In the mature leaves, moribund forms lacking cytoplasmic contents alone were observed. Similar empty structures were described in phytoplasma-infected coconut tissues from West Africa (Dabek, 1977) and in yellow leaf diseased arecanut palms in India (Nayar and Seliskar, 1978). The apparent absence of phytoplasma in mature parts and their occurrence in tender tissues suggest the movement of the organism in phloem assimilate to the 'sink' region (Parthasarathy, 1974b). Prominent ultra-structural changes noticed are the elaboration of the membrane system, presence of an unusually large number of cuneate crystalline inclusions, electron dense osmiophilic rhomboid crystals and callose deposits in sieve area (Solomon and Geetha, 1993). Comparative histopathological studies on palms with different intensities of disease revealed structural changes like disorganization and degeneration of vascular tissues and increased chromophily in phloem tissues (Govindankutty and Vellaichamy, 1983). The protophloem elements in roots and rachilla of diseased palms were often compressed and had electron dense contents indicating necrotic obliteration (Solomon and Geetha, 1993). These alterations denoted the involvement of a phloem bound pathogen.

Phytoplasmas have since then been consistently detected in phloem tissues of more than 80 diseased palms and were not observed in 75 healthy palms from disease-free area, thereby establishing the constant association of the organism with the disease. The palms examined are of various age groups, disease intensities and from different locations in Kerala and Tamil Nadu. The possible association of trypanosomatid flagellate was also

studied by examining rachilla of inflorescence of six palms each in the early, middle and advanced stages of disease. No protozoan flagellate was observed in the samples (CPCRI, 1991) and also no other biological agents such as fungi, bacteria and nematode.

Is phytoplasma transmitted through seed?

Although there is no confirmed record of transmission of phytoplasma through pollen and embryo, this aspect was studied due to its relevance to plant quarantine in the exchange of germplasm. Electron microscopic examination of pollen, embryo and sprouts of nuts collected from limited number of diseased palms did not reveal the presence of phytoplasma (CPCRI, 1988). However, Oropeza *et al*, (1995) reported detection of phytoplasma DNA by PCR from embryo of nuts collected from a few lethal yellowing diseased palms in Mexico. Similarly, Cadang-Cadang viroid is also detected in embryos and in pollen and is reported to be seed transmitted at a low rate of about one in 300 (Hanold and Randles, 1991). As these sub-microscopic agents are present in very low concentration they can escape detection through conventional microscopic techniques. More sensitive molecular diagnostic techniques need to be employed to determine the transmission of phytoplasma through pollen and seed.

Is there any simple technique for detection of phytoplasmal infection?

Since electron microscopy is laborious, time consuming and only limited area of tissue could be studied at a time, rapid histochemical staining schedules *in vogue* were tried. Dienes' staining (Deeley *et al*, 1979; Srinivasan, 1982; Razin, 1983) and fluorescent staining with DAPI (Seemuller, 1976) that bind with nucleic acid component of mycoplasmas were used for detecting phytoplasma infection in RWD affected coconut, test plants, dodder and insect vector. Free hand sections of tender rachilla and roots of diseased palms subjected to Dienes' staining exhibited abnormal bluish colouration in sieve tubes. Such staining sites were absent in tissues from healthy palms. Similarly, fixed tissues sectioned and stained with DAPI and Hoechst 33258 which binds preferentially to adenine-thymine rich phytoplasma DNA showed fluorescing areas suggestive of the accumulation of DNA in extra-nuclear sites, indicative of the presence of phytoplasma (CPCRI, 1985). Positive staining reactions are more frequent in junctions of vascular bridges and also close to the periphery of the sieve tubes (Solomon and Govindankutty, 1991). A method combining DAPI and aniline blue aids in identifying phloem tissues with ease and in finding out positive DNA accumulation in extra-nuclear positions (CPCRI, 1985). Positive extra-nuclear fluorescence also could be made out in macerated salivary gland cells of infective lace bugs and plant hoppers (CPCRI, 1990).

How does the disease spread?

Elucidation of the constant association of phytoplasma with the disease warranted the identification of the insect vector. Phytoplasmas in general are transmitted by phloem feeding insects, leaf hoppers and plant hoppers and in a few instances by psyllids. *Myndus crudus,* a cixid (plant hopper) is a vector of lethal yellowing disease of palms in Caribbean region (Howard *et al,* 1983) and *Myndus adiopodoumeensis* is a suspected vector of lethal yellowing like diseases in Africa

(Frison and Putter, 1993). However, in coconut RWD, earlier transmission experiments had implicated the lace bug, *Stephanitis typica* Distant (Tingidae), the most common heteropteran colonizing on coconut as a vector of the disease (Nagaraj and Menon, 1956; Shanta *et al.* 1964). These studies had presumed a viral etiology and needed reinvestigation in the context of the association of the phloem limited phytoplasma with the disease. Record of insects on coconut in India did not include any Auchenorrhyncha. Hence, an inventory of insects in coconut RWD affected gardens was made using various traps, sweep net collections and by direct examination of about 500 coconut seedlings over a period of two years. This study has brought out a total of 34 insects under 23 families belonging to eight orders besides eleven mite species thus necessitating a review of transmission of RWD(CPCRI 1985). A leaf hopper *Sophonia greeni* Distant and a plant hopper *Proutista moesta* (Westwood), were identified (Rajan and Mathen, 1984; 1985) in addition to the lace bug, already reported. Regarding the feeding preference of the three putative insects, leaf hopper was found on tender fronds, plant hopper on the older leaves and lace bug in increasing numbers from outer to inner whorls (CPCRI, 1985). Intact/active forms of phytoplasma being present in the tender leaves and moribund forms in the older leaves, the feeding preference of the putative insect vectors probably determines the efficiency of the insect to transmit the disease.

A survey of representative gardens in the eight districts where the disease is prevalent in a contiguous manner and in freshly reported pockets of disease incidence confirmed the presence of all the three insects, i.e. there was no disease incidence independent of the presence of these insects (CPCRI, 1985).

Of the three putative insect vectors, the phloem feeding habit of plant hoppers and leaf hoppers is known and such information was lacking for the lacebug. Experiments on lace bugs, fixed in feeding position with the stylet inserted *in situ* in the coconut leaflet, using a cold immobilisation technique followed by serial sectioning, traced the course of stylet and established that it terminated in phloem tissues. (Mathen *et al,* 1988).

The potential of lace bug to acquire phytoplasma while feeding on diseased palm was studied. Phytoplasmas were observed in the acini of salivary glands of lace bug 18-23 days after acquisition feeding and incubation on diseased palms but not from insects collected from disease-free areas nor from insects with less than 18 days exposure to diseased palms (Mathen *et al,* 1987). Although there are no reports of transmission of phytoplasma by lace bug, instances of transmission by Hemiptera other than Auchenorhynche are recorded. Paulownia witch's broom is transmitted by stink bug, *Halyomorpha mista* Uhler (Pentatomidae) (Shiozawa *et al,* 1979); Sugar beet savoy disease in North America by the tingid, *Piesma cinereum* (Say) (Piesmidae) (Proeseler, 1980).

The vector role of lacebug was proved in a transmission experiment under insect proof condition. Phytoplasma could be observed in all the four coconut seedlings (2 yr old) inoculated with infective lace bugs in nine to twenty seventh month of the start of the experiment. Two of the

seedlings exhibited flaccidity, the decisive diagnostic symptom of the disease by the seventeenth month. None of the uninoculated control seedlings neither exhibited visual symptoms nor had phytoplasmas in the phloem tissues (Mathen *et al,* 1990).

Additional evidences supporting the vector role of lace-bug have also been documented. The lace bugs colonizing in large numbers in the inner leaves of the crown where active forms of the organism are generally found (Mathen *et al,* 1969), the diseased palms lodging four times higher the number of lace bugs than in healthy palms (Mathen, 1982) and a direct linear correlation between the number of bugs in a palm and incidence of disease added further significance to its vector role (Mathen, 1985).

Phytoplasmas have also been observed in the salivary glands of *P. moesta* examined more than 30 days after acquisition feeding (CPCRI, 1991). The vector role of this insect was also studied in a transmission experiment. Phytoplasma could be observed in six plant hopper-inoculated 2 yr. old coconut seedlings from 5-24 months of inoculation. Five of the seedlings have shown 'flaccidity', the characteristic symptom of the disease (CPCRI, 1997) thus confirming the vector role of *P. moesta.*

Can the disease be controlled through insecticide application?

Field experiments were conducted to study the effect of foliar/soil application of insecticides and plant products on the incidence of the disease. Disease incidence was recorded even in plants sprayed fortnightly with insecticides. No significant difference was observed between treatment and control (CPCRI, 1993, 1997). Similar results were recorded in lethal yellowing disease in Florida. Biweekly spraying of insecticides resulted only in slight reduction in vector population and in spread of LY (Howard and Mc Coy, 1980; Howard and Barrant, 1989). Howard (1987) is of the view that the insecticides currently available are not persistent and thus treated palms are quickly reinfested resulting in disease contraction

The perennial nature of crop, persistent mode of transmission, and the presence of the insect vectors almost throughout the year in coconut may be the reasons for the non-effectiveness of the insecticides in preventing fresh incidence of disease. Large scale chemical treatments at frequent intervals also would be costly and can pose environmental hazard.

Experimental transmission of the disease to *Catharanthus roseus* G. Don, a phytoplasma indicator host was tried using dodder. Species of *Cuscuta, C.campestris, C. chinensis* and *C. subinclusa* although established haustoria on coconut leaflets, failed to develop intimate haustorial connections with coconut leaf vasculature. Failure of Tsai (1983) to transmit LY to coconut using *C. campestris* could be due to the lack of establishment of vascular connection. However, the RWD phytoplasma could be transmitted from coconut to periwinkle using the dodder laurel, *Cassytha filiformis.* Within three weeks of establishment of haustorial connections, chlorotic spots appeared in the interveinal areas and vein endings of fully opened leaves of the periwinkle test plants. Presence of phytoplasma in the diseased source palm, dodder laurel bridge and the recipient periwinkle was

confirmed by electron microscopy (Sasikala *et al,* 1988).

Can the phytoplasma be cultured?

Although the phytoplasma associated with RWD had been transmitted through insect vectors to coconut seedlings and to periwinkle through the dodder laurel, culturing of the organism in cell-free medium and proving its pathogenicity is one of the basic tenets of Koch's postulates. Concerted effort was made to culture the organism from rachilla of unopened inflorescence, spear leaf, mid vein, root tips and vascular sap from RWD affected palms, petiole and midvein of symptomatic periwinkle and infective lace bugs in several media, based upon a rationale developed by Jones *et al,* (1977). Serum enriched media supplemented with nucleotides, nucleosides, aminoacids, nucleic acid precursors, co-factors and chemicals which reduce the oxidation reduction potential too were tried. Likewise, media in which serum was substituted with other enrichments were also used (CPCRI, 1988, 1989). A number of media (totalling 40) in liquid, semi solid and solid states with various combinations of nutrients and cultural conditions were also tried but not with success (Srinivasan and Solomon, 1998a).

Lee and Davis (1986) had suggested a novel approach to development of media using coconut phloem sap as a base for culturing of phytoplasma. Phloem sap as such or supplemented with serum is found to be an ideal medium for culturing fastidious organisms such as *Acholeplasma laidlawii, Mycoplasma fermentans, Spiroplasma citri* and *Phytomonas davidi* (Eden-Green and Waters 1982, Mc Coy 1976, 1977, 1978). Srinivasan and Solomon (1998a) also obtained growth of *Mycoplasma bovis, Acholeplasma laidlawii* and sesamum phyllody *spiroplasma* in enriched phloem sap and nut water media. A method for aseptic collection of fresh and unmodified vascular sap from inflorescence of coconut palm was standardized and the biochemical constituents of vascular sap from apparently healthy and diseased palms analysed (Rajagopal *et al,* 1988). The lower content of the major biochemical constituents in sap from diseased palms could possibly be due to the higher rate of utilisation of these by phytoplasmas for their growth and multiplication (Chempakam and Rajagopal, 1989). Nevertheless, the data provided a rationale for formulation of new media. Freshly collected vascular sap from apparently healthy palms as such or supplemented with various nutrients was tried as a potential medium for culturing. Coconut water from nuts of healthy palms in different stages of maturity was also used as basal constituent of certain media. The intra-phloemic phytoplasma, however, was not found to grow in any of the media (Srinivasan and Solomon, 1998a). Phytoplasmas as a group have not been cultured so far in cell-free medium. Only spiroplasmas have been cultured *in vitro* (*e.g.* Sesamum phyllody *spiroplasma*; Srinivasan and Solomon, 1991).

Certain fastidious spiroplasmas such as suckling mouse cataract agent (SMCA) in ticks could be successfully cultured in cell-free media after its cultivation in embryonated hen's eggs (Tully *et al,* 1976, 1977). Hence attempts were made to culture the RWD phytoplasma in chick embryo *in vivo* system also using different procedures of inoculation and incubating under varying cultural conditions (Srinivasan

and Solomon, 1998b). Although in an identical study the spiroplasma associated with phyllody disease in sesamum could be cultured in chick embryos (Srinivasan and Solomon, 1992), the RWD phytoplasma had failed to grow in this *in vivo* system also, confirming its poor adaptability to environments outside the host/vector.

During the numerous attempts to culture the RWD agent from host, indicator plants and vector into cell-free media and chick embryos neither *Spiroplasma* nor *Acholeplasma* was encountered. Considerable amount of efforts were taken to culture the organism *in vitro*. The study has established the RWD organism to be a filterable agent or rigid fastidious body that cannot be cultured in cell-free media. These investigations have helped in changing the name of the causative agent of RWD from MLOs to that of Phytoplasma (Albanese *et al,* 1994).

Another approach in culturing RWD phytoplasma was aimed at the maintenance of phytoplasmas in plant tissues micropropagated *in vitro* in tissue culture media. Tender leaf explants of diseased palms were inoculated to modified MS medium. About 10% of the cultures developed root initials in four weeks. The roots emerging from the leaf explants could be maintained in culture for eighteen months. Phytoplasma could be observed in the roots in culture upto nine months. Thereafter, moribund forms alone were evident (Solomon and Govindankutty, 1991). A number of phytoplasmas which are not cultured otherwise in cell-free media have been successfully maintained for long period in plant tissues micropropagated *in vitro* (Cousin *et al,* 1990; Bertaccini *et al.* 1992; Davis and Clark, 1994; Jarausch *et al,* 1996). Palms, as such being recalcitrant to tissue culture, and the intra-phloemic phytoplasma being fastidious, difficulty in culturing the organism is not unexpected. Hence, unconventional methods have to be tried in the cultivation of the organism. The phytoplasma seem to be more exacting in their requirements. The plant/insect tissue culture system may be refined and the organism could possibly be allowed to concentrate in such *in vivo* systems. Enzymatic maceration and separation of sieve elements of diseased palm tissues and maintenance of phytoplasmas in viable culture and slow release of these into culture medium using refined techniques are likely to be the other alternatives.

Does the disease respond to antibiotic therapy?

Non-cultivable nature of phytoplasma in cell-free medium is a strong limiting factor in testing its pathogenicity and fulfilling the Koch's postulates. Characteristically, these prokaryotes lacking a cell wall, and delimited by cell membrane are insensitive to penicillin, though sensitive to tetracycline group of antibiotics. This differential chemotherapeutic response is used as an adjunct to provide circumstantial evidence for the etiology of phytoplasma induced diseases. To assess the efficacy of any chemical on the disease, an effective method of application into the plant system is a pre-requisite. The chemical administered should reach the target site in an unaltered state within a reasonable period of time. A pneumatic pressure injector fabricated indigenously (Pillai and Raju, 1985) was found to be ideal for the purpose. The uptake

and translocation of the antibiotic was studied using a tracking dye, Rhodamin B and through bioassay using *Bacillus cereus* sub-sp. *mycoides* as the test organism. The antibiotic Oxytetracycline hydrochloride (OTC) could be detected in the foliage within 24h of its application. It reached maximum concentration in the foliage in 5-6 days after injection. Maximum OTC activity 3-11 µg/g fresh weight was found in leaf no. 11. Very little OTC acti.ity was noticed in the non tranpiring tissues of inflorescence, roots and spear leaf (Chowdappa *et al,* 1989). Mc Coy (1976) recorded similar results in lethal yellowing diseased mature coconut palm trunks injected with OTC by the pressure tank method. It was observed that with the onset of senescence, the antibiotic gets redistributed from older leaves to the most actively transpiring leaves. Detectable levels of OTC was observed for more than 12 weeks in the foliage (Chowdappa *et al,* 1989).

The standardization of the most effective method for infusing the antibiotic into the plant system and information generated on the uptake, translocation and retention of OTC in different parts of the plant aided in scheduling a field trial. The experiment was conducted in two locations on ninety RWD affected West Coast Tall palms of 14-16 years. The disease index score of the experimental palms, varied from 20-30. Fifteen palms each were injected with 1,2,3 and 6g active ingredient of Oxytetracycline-HCl (OTC) (Terramycin 20% soluble powder of M/s Pfizer Ltd., India) and 3,000,000 units of pencillin and distilled water. The palms were reinjected at quarterly intervals and the foliar condition indexed twice a year. The experiment was continued for a period of 36 months, the period required for complete replacement of the canopy with fresh leaves. Results of the study indicate remission of symptoms in 53.3% of palms treated with 3g and in 53.9% of palms injected with 6g ai of OTC. In contrast, palms treated with Penicillin and distilled water deteriorated over the pre treatment condition (Pillai *et al.* 1991). In a similar study on lethal yellowing diseased palms in Florida, Mc Coy (1975) obtained remission of symptoms only in 50% of the treated palms. The response of a significantly higher number of RWD affected palms to OTC treatment is indicative of the involvement of phytoplasma, a wall-less mollicute that is sensitive to tetracycline. The deterioration of palms treated with Penicillin, which is effective only against walled bacteria, further substantiates the above findings. The differential chemotherapeutic effect recorded has thus conclusively established the involvement of phytoplasma as the causative agent of the disease.

To obtain permanent control of phytoplasma disease by chemotherapy, the compounds should be evenly distributed within the plant and should be present in all plant parts at a concentration high enough to kill the phytoplasma. Schaper and Seemuller (1982) are of the view that these requirements cannot be achieved with the present application methods and chemicals. Distribution of the OTC being very poor in nontranspiring tissues of the inflorescence, root and spear leaf that contain maximum concentration of phytoplasma in the plant, the chemical may not be able to exert a complete check on the multiplication of the organism. Moreover, the pattern and speed of distribution of OTC indicates a xylem transport and

further radial distribution though the inter-connecting vascular net work (Zimmermann and Tomlinson 1974). Phytoplasma being phloem limited, also may account for the partial remission observed in antibiotic-treated palms. Schaper and Seemuller (1982) concluded that the survival of the causal agent in the roots seems to be the main reason for the failure of the tetracycline treatments to give long lasting control. After the concentration of the antibiotic has fallen below the threshold level of activity in the crown, it can be reinvaded by phytoplasma from root and other non-transpiring plant parts. The results on partial remission of symptoms achieved should therefore, be viewed in the light of the above findings. Field application of OTC, either as prophylactic or as curative measure for the control of the disease cannot be recommended as the antibiotic needs to be administered repeatedly and remission of symptom is only temporary. Prohibitive cost of the antibiotic and the caution against its indiscriminate use for treating any plant disease are other limitations against its usage.

Are there any resistant/tolerant varieties?

To identify genotypes resistant/tolerant to the RWD, 63 cultivars and 32 hybrid combinations were evaluated in the cultivators' gardens since 1972. None of the cultivars/hybrids were found to be resistant. However, the cultivar Chowghat Green Dwarf (CGD) was found to have field resistance of over 90% to the disease (CPCRI, 1972). Indian Green Dwarf had shown 89% resistance to lethal yellowing disease of coconut in Jamaica, a disease induced by phytoplasma (Coconut Industry Board, Jamaica, 1983, 1984). The level of resistance of Green Dwarf to LY is said to be equal to that of Malayan Dwarf (Schuiling *et al,* 1992).

Currently, healthy CGD palms are being used to cross with RWD-free elite West Coast Tall (WCT) palms identified in hot spots of four districts - Alleppey, Quilon, Kottayam and Pathanamthitta to develop disease resistant hybrids with desirable yield potential (Nair *et al.* 1996). The hybrid progenies have been planted in a phased manner since 1991, in diseased gardens in the endemic tract to assess their performance. Of the 31 hybrids planted in 1991, five palms are showing the symptoms of disease. The performance of the progenies is to be further monitored for disease incidence and yield. Concurrently, disease-free WCT palms are also selfed/*inter se* crossed for evolving a disease-free tall population.

Exotic germplasm collections made from South Pacific Ocean Islands in 1981 comprising 24 accessions (Rao *et al.* 1993) have been planted in the offshore quarantine station in Andamans with a view to screening their progenies and different cross combinations against the disease in future. A second collection of fifteen accessions in the form of embryos have been made from Indian Ocean Islands in 1997 for the same purpose (CPCRI News Letter, 1997).

Can the RWD be managed?

One of the redeeming features of the RWD is that it is not lethal, but a slow declining malady that responds to ideal management practices. Removal of diseased palms in the mildly affected area arrests further spread of the disease. These two features aided in formulating separate disease management strategies, one for the heavily

diseased area and another for the mildly affected area.

Integrated disease management strategy for the heavily affected areas include the removal of all disease advanced and senile palms and juvenile diseased palms, replanting with quality seedlings raised from hot spot areas, spraying the crown with insecticides before felling to kill the infectious vectors, application of balanced dose of fertilizers, addition of organic matter, raising green manure crops in the basin and their incorporation, irrigation during summer months, control of leaf rot and major pests, providing proper drainage and restructuring the canopy of other perennial crops to provide maximum sunlight for coconut and adopting inter/mixed cropping (Bavappa *et al,* 1986; Muralidharan *et al,* 1991).

Mixed farming in diseased gardens by cultivating fodder crops in the interspaces, maintaining milch cows and recycling of organic wastes has helped in increasing the yield of palms by 26% over a period of five years (Sahasranaman *et al,* 1983). Significant increase in soil fertility status was also observed.

Mixed cropping with cocoa increased the overall yield of coconuts by 30% and slowed down the deterioration of palms (Bavappa *et al,* 1982). Similarly, cultivation of tapioca; elephant foot yam and yam in the interspaces of disease affected gardens for a period of 3 years increased the nut yield by 4.96, 15.57 and 8.07% respectively (Menon and Nair, 1979). Irrigation of palms during summer months is also found beneficial in improving both the health and yield of palms (Rajagopal *et al.* 1987). Adoption of integrated management practices not only enhanced the yield of palms by 23.4 nuts/palm/year but also improved the overall condition of the palms. This response was noticed mainly in palms in the early stage of disease and was not evident in palms in the advanced stage (Muralidharan *et al,* 1986).

Recommendation for the mildly affected area comprises the removal of all diseased palms to arrest further spread of the disease. In a large-scale field experiment conducted during 1979-1989, a total of 730 diseased palms in 341 gardens of 10 villages in Trichur district were removed. This brought down the recurrence of the disease to 21 palms spread over 15 gardens in 3 villages. Similarly, removal of three diseased palms in Vallam village in Shencottah, Tamil Nadu during 1979 prevented recurrence of disease in the area (Bavappa *et al,* 1986). Surveillance and removal of diseased palms is implemented in Coimbatore and Kanyakumari districts of Tamil Nadu to arrest the spread of disease to newer areas.

Discussion

Phytoplasmal etiology of the coconut RWD has been conclusively proved by the establishment of constant association of the organism with the disease and its absence in healthy palms from disease-free area, detection of phytoplasma in the salivary glands of infective lace bug and plant hopper and experimental transmission of the disease from diseased to healthy coconut seedlings through insect vectors, reproduction of symptoms and confirmation of phytoplasma through EM, non-cultivable nature in cell-free media, experimental transmission from

coconut to periwinkle, maintenance in plant tissues micropropagated *in vitro* and remission of symptoms with OTC treatment.

Generally, the major host response to phytoplasma infection in most diseases is stunting and slow decline. However, a few phytoplasma diseases are lethal, resulting in devastating epidemics such as lethal yellowing of coconut, sandal spike and elm yellows (Bertaccini *et al.* 1996). Stubborn infected citrus trees rarely die no matter how severe the symptoms are, but it causes sudden wilting and death of herbaceous plants like pea, bean and periwinkle (Gumpf and Calavan, 1981). Most commonly phytoplasmas cause diseases that debilitate infected trees, resulting in gradual decline and reduction in quantity and quality of fruits (Seliskar and Wilson, 1981). The trend in RWD of coconut is identical, with a slow decline resulting in reduction in quantity and quality of the produce. For example, in ash yellows although mature trees show slow growth and decline, rapid decline is noticed only in seedlings and saplings (Sinclair and Griffith, 1994). Similarly, coconut seedlings contracting the RWD disease in the prebearing age, decline rapidly and may not come to bearing (Ramadasan *et al,* 1971). Unlike dicots, coconut being a monocot and lacking a cambium, having a solitary growing point can respond to only a limited range of symptom expression to pathological disturbances (Maramorosch and Hunt, 1981).

Future thrust

Molecular diagnostic techniques such as polymerase chain reaction (PCR) and use of restriction fragment length polymorphism (RFLP) may be standardized as sensitive tools for characterization of phytoplasma at molecular level and its detection in coconut, other palms, weeds and putative insects. This could also help in the selection of disease-free palms for breeding for disease resistance studies and also to screen seedlings in the nursery. RWD phytoplasma may be purified, characterized, compared with other phytoplasmas and ascribed to its taxonomic position. Plant products inimical to phytoplasma and chemicals capable of inducing host resistance may be tried.

As breeding for disease resistance is important, more number of disease-free mother palms may be selected and bred rigorously for resistance/ tolerance.

The disease management strategies for the heavily diseased area and the mildly affected area must be strictly implemented to get better returns from unit area and also to arrest the spread of the disease.

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