ROOT (WILT) DISEASE OF COCONUT— CURRENT STATUS

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ABSTRACT

Root (wilt) disease of coconut reported over a century ago in Kerala, South India, is a non-lethal but declining malady. The annual loss due to the disease is estimated to be about 968 million nuts. The cause of the disease remained uncertain till recently. Recent studies have conclusively ruled out involvement of biological agents, nutritional and physiological factors as disease incitant(s). Identification of a phloem bound mollicute, its insect vector and the evidences accrued in favour of the mycoplasmal etiology are discussed.

INTRODUCTION

Coconut root (wilt) disease has been the subject matter of a number of treatise in the past (Jayasankar and Bavappa, 1986; Nair *et al.,* 1991). The scope of this paper is to give an overview on the current status of the etiology of the disease. Root (wilt) disease was first reported after the great floods of 1882 in three independent locations, each about 50 km apart in the erstwhile state of Travancore (Butler, 1908; Kunjan Pillai, 1911; Varghese, 1934). Since then it has spread from the original foci of infection. According to a survey conducted during 1984/85, the disease is prevalent in more or less a contiguous manner in 0.41 million ha in the eight southern districts of Kerala (Anonymous, 1985). It is also noticed in few isolated pockets in the northern districts of the state (Radha *et al.,* 1985) and in the bordering districts of Tamil Nadu. The intensity of the disease in the contiguous diseased tract ranged between 1.52 per cent in Thiruvananthapuram district and 75.63 per cent in Kottayam district. The annual loss due to the disease is estimated to be about 968 million nuts. The disease is non-lethal but debilitating and palms of all age groups are affected. Delayed flowering and reduction in yield are observed in palms contracting the disease in the pre-bearing age (Ramadasan *et al.,* 1971). The disease occurs in

all major soil types. However, the spread is faster in sandy, sandy loam, alluvial and in heavy textured clayey soils than in laterites. The disease incidence is relatively higher in water logged low lying areas adjacent to rivers and canals and in *Kari* soils (Pillai *et al.*, 1973).

Symptoms

The most consistent and diagnostic symptom of the disease is the characteristic ribbing of leaflets termed flaccidity (Radha and Lal, 1972). Foliar yellowing and marginal necrosis are the other associated symptoms (Fig. 73.1). Yellowing is virtually absent in young palms where flaccidity is the only symptom (Radha and Lal, 1972). George and Radha (1973) developed an indexing system for quantifying the disease, giving due weightage to intensity and frequency of occurrence of these three major symptoms.

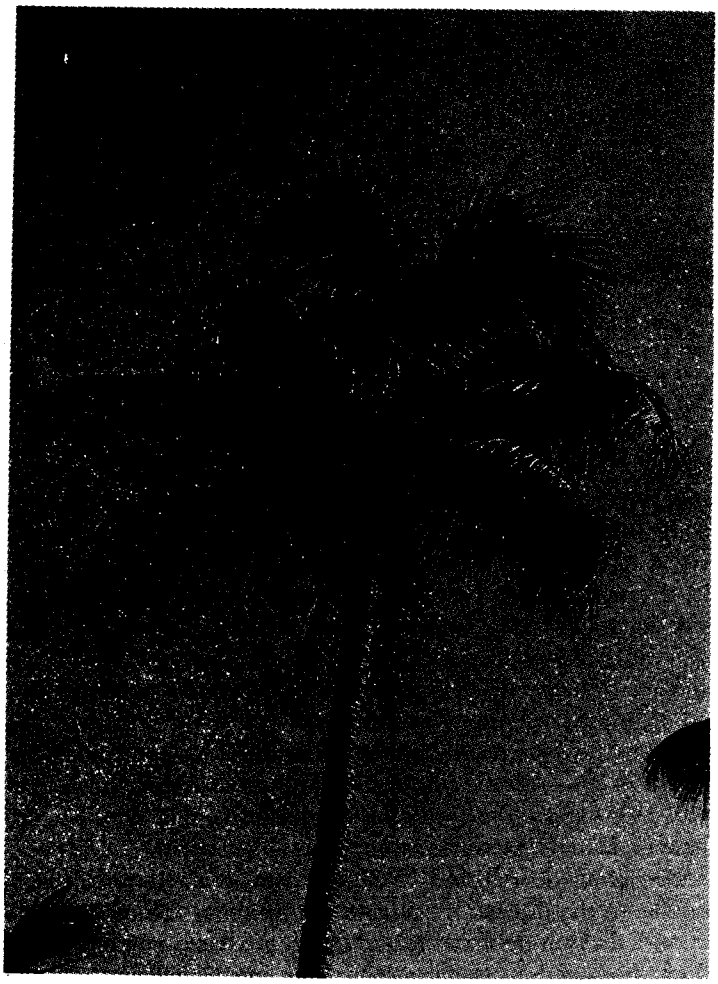

Fig. 73.1: Coconut palm showing flaccidity and necrosis of leaflets.

Rotting of roots was once considered as a major symptom (Butler, 1908; Menon and Nair, 1949; Menon and Pandalai, 1959; Michael, 1964). However, this could not be substantiated in later studies (Nagaraj and Menon, 1955; Lal, 1969; Joseph and Jayasankar, 1981). Drying up of spathe and necrosis of spikelets extending from tip downwards in tender unopened inflorescence was noticed in certain cases (Menon and Pandalai, 1959; Maramorosch, 1964). A high percentage of pollen produced was either sterile or with low viability (Varkey and Davis, 1960). Similarly, meiotic irregularities were also observed in diseased palms (Nambiar and Prasannakumari, 1964). Abnormal shedding of female flowers and immature nuts and lack of ability to produce female flowers significantly affected the palms' productivity (Varghese 1934; Menon and Pandalai, 1959). Kernel of nuts from diseased palms does not dry normally and remains flexible (Menon and Nair, 1951; Maramorosch, 1964).

Leaf rot disease caused by certain fungi occurs superimposed on about 30 per cent of the root (wilt) affected palms bringing about a rapid decline in yield (Anonymous, 1989a). However, this could be controlled to a greater extent with sequential spraying of fungicides (Anonymous, 1986).

Diagnosis

Field identification of diseased palms is by and large through visual symptoms. The need for a reliable diagnostic test, preferably one which could detect the disease status before the expression of foliar symptoms, led to exploring of several tests (Sasikala *et al.,* 1991). A sero-diagnostic test (Solomon *et al.,* 1983) and a physiological test (Rajagopal and Amma, 1989) based on differential stomatal resistance have been found to be reliable. With these tests, the disease condition could be diagnosed six to 24 months before the expression of foliar symptoms (Rajagopal *et al.,* 1988b).

ETIOLOGY

Biological agents

Etiology of the disease remained an enigma until recently. A number of biological agents such as fungi, bacteria and nematodes were reported to be associated with the disease. However, pathogenicity studies with these organisms singly and in combination failed to reproduce the symptoms of the disease, thereby, ruling out their involvement in disease causation (Joseph and Lily, 1991; Jayasankar and George, 1991; Sosamma and Koshy, 1991).

Physiological Factors and Nutrients

Physiological and biochemical changes observed in diseased palms are indicative of a pathogen altered host metabolism than of a physiological disorder (Mathew *et al.,* 1991). Similarly, an extensive analysis of soil and leaf tissues of palms in healthy and diseased tracts and various fertiliser trials conducted clearly ruled out the involvement of any major or micronutrients in the incidence of the disease (Cecil and Amma, 1991).

Virus

Association of a submicroscopic agent possibly a virus or virus-like agent was postulated by a number of workers (Nagaraj and Menon, 1956; Shanta and Menon, 1961; Holmes, 1965; Summanwar *et al.,* 1969; Maramorosch and Konda, 1977). However, no virus could either be isolated or consistently observed in tissues of diseased palms (Solomon and Sasikala, 1981). Polyacrylamide gel electrophoretic analysis of isolated nucleic acids from diseased palms also excluded the association of any viroid type pathogen with the disease (Randles and Hatta, 1980).

Mycoplasma-like Organisms (MLOs)

Comparative histopathological studies on tissues of healthy and diseased palms revealed structural alterations such as disorganisation and degeneration of vascular tissues, increased chromophily and necrotic obliteration in the latter (Govindankutty and Vellaichamy, 1983). These observations prompted detailed ultrastructural studies of the vascular tissues. Electron microscopic examination of juvenile tissues like submeristem, petiole of developing leaves, rachilla of unopened inflorescence and root tips of diseased palms showed the presence of a phloem bound mollicute—the mycoplasma-like organism (MLO) (Solomon *et al.,* 1983). These prokaryotes are bound by a trilamellar unit membrane and contain DNA strands and ribosomes (Fig. 73.2). Pleomorphic forms varying from circular to oval and occasionally beaded or filamentous ones are also observed. The coccoid forms are in the size range of 250 to 400 nm. They are found in increasing numbers in the 'sink' region. Degenerated or moribund forms are often observed in mature tissues (Solomon *et al.,* 1987). Constant association of the organism with the disease

Fig. 73.2: Mycoplasma-like organisms in sieve tubes of tender petiole from root (wilt) diseased coconut palm.

has since been established with the finding of MLOs in tissues of 70 diseased palms and their absence in 50 healthy palms studied. Intracellular presence of the organism, their absence in healthy palms and the structural changes observed further adduced to its etiological role. Interestingly, none of the other biological agents reported associated with the disease could be observed in the vascular tissues of the palms studied.

Histological staining techniques for visualising MLOs under optical microscope have also been standardised. Free hand sections of tender rachilla and roots of diseased palms subjected to Dienes' staining exhibited abnormal bluish colouration in sieve tubes. Tissues of healthy palms were devoid of such staining sites. Similarly, fixed tissues sectioned and stained with fluorochrome, 4,6-diamidino-2 phenyl indole 2 HCl (DAPI) and Hoechst 33258 showed fluorescing areas in the sieve cells suggestive of the accumulation of DNA in extra-nuclear sites indicating the presence of MLOs (Solomon *et al.*, 1987). Such positive reactions are more frequent in junctions of vascular bridges and also close to the sieve plate.

Elucidation of the constant association of MLOs with the disease warranted identification of the insect vector. Lace bug—*Stephanitis typica* Distant (Tingidae)—being the single major group of insect on coconut and based on transmission experiments was presumed to be the vector of the disease (Nagaraj and Menon, 1956; Shanta *et al.*, 1964). Since MLOs are generally transmitted by leaf hoppers, plant hoppers and in a few instances by psyllids, the vector role of lace bugs needed reinvestigation. A record of insects on coconut in India did not include any belonging to the conventional mycoplasma transmitting group—Auchenorhyncha. An inventory of all insects of transmission importance was made through various trapping aids (sticky, rotary flight, suction and light traps) supplemented with direct observation of over 200 seedlings for a period of two years. This led to the identification of a leaf hopper, *Sophonia greeni* (Distant) and a plant hopper, *Proutista moesta* (Westwood) (Rajan and Mathen, 1984; 1985). A rapid survey of representative gardens in eight districts where the disease occurs contiguously revealed that the disease does not exist independent of these three insects.

A study of the feeding habit of lace bug by cold immobilisation of stylet fixed *in situ* in coconut pinnae followed by serial sectioning revealed its termination in phloem, thereby indicating the phloem feeding nature (Mathen *et al.*, 1988). The potential of these putative insects to acquire MLOs while feeding on diseased palms was further studied. MLOs were observed in salivary glands and brain tissues of lace bugs offered acquisition and incubation period (A + I) of 18 to 23 days on diseased palms. Lace bugs from disease free areas and insects offering A + I less than 18 days were found to be free of the organism (Mathen *et al.*, 1987). Recently, the MLOs have also been located in plant hoppers given A + I of over 37 days (Anonymous, 1990).

Insect Transmission

The vector role of lace bug was studied in a transmission experiment. Eight West Coast Tall, two-year-old coconut seedlings obtained from a disease

free area and planted in methyl bromide fumigated soil held in field tanks of 1.8 × 1.8 × x 1.2 m and protected with slanting insect proof cages of 3.7 m were used for the experiment (Fig. 73.3). Four of the seedlings were inoculated with lace bugs offered five days acquisition feeding on diseased palms and insects offered 18 days A + I as detailed in Table 73.1. Four uninoculated plants grown in insect proof cages were maintained as control. Nine months after the first inoculation, three out of four seedlings showed strong positive serological reaction and faint reaction in the fourth indicating disease contraction. Similar results were also obtained in Dienes' and fluorescence staining. EM observation of the root tissues confirmed the presence of MLOs in all four seedlings between nine and 27 months after the first inoculation. Flaccidity of leaflets, the decisive and diagnostic symptom of the disease, was evident in two of the seedlings by the seventeenth month. None of the uninoculated control seedlings either exhibited visual symptoms or showed the presence of MLOs (Mathen *et al.*, 1990).

Table 73.1: Number of lace bugs inoculated on experimental coconut seedlings in field cages numbered 3, 4, 9 and 10

Date	*5 days' acquisition*				*Total*	*5 days' acquisition plus 13 days' incubation*				*Total*
31 Dec 1985	184	180	180	174	718					
31 Mar 1986	200	196	202	180	778					
30 Jun 1986	631	637	639	664	2571					
Total	1015	1013	1021	1018	4067					
30 Sep 1986						626	621	616	595	2458
31 Dec 1986						427	431	434	458	1750
31 Mar 1987						90	76	80	76	322
Total						1143	1128	1130	1129	4530
30 Jun 1987	100	101	106	97	404					
30 Sep 1987	278	285	257	287	1107					
30 Nov 1987	–	705	–	706	1411					
Total	378	1091	363	1090	2922					
Grand total	1393	2104	1384	2108	6989					

Apart from the direct evidence emerging out of the transmission experiment, a number of indirect evidences have also accrued in support of the vector role of lace bug. They are found colonising in increasing number towards the inner leaves of the crown where active forms of the organism are generally found (Mathen *et al.*, 1969). The number of lace bugs on diseased palms is found to be four times the number in healthy palms (Mathen, 1982). After monitoring of lace bug population for two years in about 700 juvenile palms, Mathen (1985) reported a direct linear correlation between the number of insects colonising the palms and a fresh incidence of disease. Field

experiments are in progress to see whether the control of aerial insects can regulate the fresh incidence of disease.

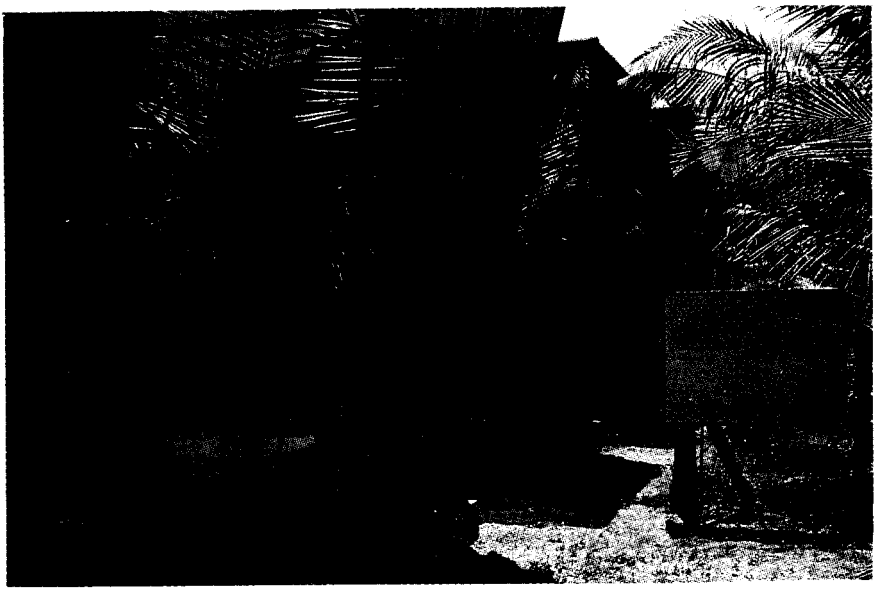

Fig. 73.3: Insect proof cages for transmission experiment.

Dodder Transmission

Experimental transmission with dodder species was also performed. The conventional dodder, *Cuscuta* species although established on coconut foliage did not develop intimate haustorial connection. *Cassytha filiformis*, a member of Lauraceae established strong vascular connections on coconut pinnae. Dodder laurels established on field palms were bridged to potted periwinkles in nylon netted cages. Within three weeks of haustorial connections, the periwinkle exhibited chlorotic spots in the interveinal areas and at vein endings of fully opened leaves. Ultrathin sections of the leaves of diseased source palm, connecting dodder, laurel and recipient periwinkle showed the presence of MLOs. The organism could also be serially transmitted from periwinkle to periwinkle (Sasikala *et al.*, 1988).

Culturing

Although transmission through insect and dodder have been achieved, culturing of MLO is still considered to be vital to fulfil the requirements of Koch's postulate. Mycoplasma being confined to phloem, a medium simulating the physicochemical environment of the phloem may be necessary for culturing of the organism. Phloem sap as such or supplemented with serum has been found to be an ideal medium for culturing of fastidious organisms such as *Acholeplasma laidlawii, Mycoplasma fermentans, Spiroplasma citri*, and *Phytomonas davidi* (Eden-Green and Waters, 1982; McCoy, 1976; 1977; 1978). Rajagopal *et al.* (1988a) standardised a method for aseptic collection of vascular

sap in ice-packed vacuum flask and analysed the constituents of the sap. The vascular sap from apparently healthy palms supplemented with serum was utilised for *in vitro* culturing of the organism. In addition over 40 different media with various combinations of nutrients and other cultural conditions were used for culturing of the organism from tissues of diseased palms, symptomatic periwinkle and infective lace bug. Culturing in chick embryos was also tried. However, the organism could not be cultivated (Anonymous, 1989b). Currently, the possibilities of long-term maintenance of the organism in explants of diseased palms and selected organs of infective lace bugs are being explored. Preliminary studies indicate that the mollicutes could be maintained in tissue cultured explants for six to eight weeks (Anonymous, 1989b).

Chemotherapy

Since MLOs are not amenable to culturing *in vitro*, differential chemotherapy is universally advocated as the best tool to provide circumstantial evidence for the mycoplasmal etiology of a disease. To ensure that the antibiotic reaches the target site in an unaltered state within a reasonable period, a number of injection devices were tried. An indigenously fabricated pneumatic pressure injector was found ideal for the purpose (Pillai and Raju, 1985). The antibiotic reached the foliage within 24 h of its application and traces of the chemical persisted up to 12 weeks as confirmed by bioassay using *Bacillus cereus* sub sp. *mycoides* as the test organism. Results of a field trial with four concentrations of oxytetracycline hydrochloride (OTC), a single concentration of Penicillin and distilled water control clearly indicated remission of symptoms in palms treated with 3 g and 6 g ai of OTC. Contrastingly, palms in both Penicillin and distilled water controls deteriorated over the pre-treatment condition (Pillai *et al.*, 1991). Although field application of OTC cannot be recommended either for the control of the disease or as a prophylactic measure, the results have nevertheless given convincing evidence to the mycoplasma etiology of the disease.

Screening of Coconut Germplasm

Field evaluation of 45 cultivars and 62 hybrid combinations in progress since 1972 indicated that none of the cultivars/hybrids is resistant to the disease. However, the hybrid Chowghat Orange Dwarf (COD) × West Coast Tall (WCT) under ideal management yielded a higher number of nuts compared to WCT of identical age in the initial years of production (Anonymous, 1986).

Breeding for Disease Resistance

An intensive survey in the heavily diseased area (hot spot) has been conducted to locate disease free high-yielding palms above the age of 35 years. Palms finally selected after subjecting to serological and physiological tests are being used as parents in the breeding programme.

Twenty-four exotic accessions from South Pacific Ocean Islands have been planted in the World Coconut Germplasm Centre at Sipighat in the Andamans for producing *inter se* and selfed nuts for screening against the disease.

Future Thrust

1) Assessing the vector role of plant hopper through transmission experiment under controlled condition.

2) Intensifying the breeding programme to evolve identical plant type resistant/tolerant to disease.

3) Developing DNA hybridisation probes for detection of MLOs infection and integrated management of diseased gardens to obtain optimum yield.

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DISCUSSION

P. Rethinam: Can you indicate the number of diseased palms tested for MLOs and for the protozoan flagellate studies?

J.J. Solomon: Seventy diseased palms and 50 healthy palms were examined for MLOs. The palms sampled were from different locations, intensities of disease and age groups. For protozoan flagellates six palms each in early, middle and advanced stages of disease were examined.

Carlos Oropeza: Have you tried MLO transmission from *Catharanthus roseus* to coconut?

J.J. Solomon: No. We have not so far tried MLO transmission from *Catharanthus roseus* to coconut. However, we are planning to do transmission studies between diseased coconut to healthy coconuts and from *Catharanthus* to coconut.

H.C. Harries: Is it possible for viroid or virus infection to predispose palms to MLO infection which they might otherwise resist?

J.J. Solomon: Possible association of viroids with coconut root(wilt) disease was investigated in collaboration with Dr. J.W. Randles of Australia. PAGE analysis of isolated nucleic acids and molecular hybridisation techniques have ruled out the involvement of viroids with the disease. EM examination of purified samples and thin sections of various plant parts have ruled out the involvement of viruses with the disease. Hence the question of predisposition of palms to MLO infection by viroid or virus does not arise.