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Current Status of Research on Root (Wilt) Disease of Coconut in India

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ABSTRACT

Root (wilt) disease, a non-lethal but debilitating malady of coconut reported over a century ago, now occurs throughout 0.41 million ha in Kerala, South India. It causes annual losses of about 968 million nuts; palms of all age groups are affected. Investigations on the etiology of the disease have failed to produce evidence for the involvement of fungi, bacteria, nematodes, viruses, viroids, protozoa, or nutritional and physiological factors. Ultrastructural studies of juvenile tissues of diseased palms revealed phytoplasmas (mycoplasma-like organisms, MLOs) in phloem sieve tubes. Constant association of phytoplasmas with the disease has been established. Dienes' and fluorescent staining schedules to detect phytoplasmas were standardized. A sero-diagnostic and a physiological test have been developed for early detection of the disease.

Surveys of putative insect vectors on coconut identified a leafhopper, *Sophonia greeni*, a planthopper, *Proutista moesta*, and a lace-bug, *Stephanitis typica* Tingidae, as putative vectors. Phytoplasmas have been detected in the salivary glands of the lace-bug and planthopper following acquisition feeding and incubation on diseased palms. The vector role of the lace-bug has been established; transmission of the disease by the planthopper is under experimentation. Experimental transmission of the disease from coconut to periwinkle, *Catharanthus roseus*, was achieved through dodder laurel, *Cassytha filiformis*. Vascular sap collected from healthy and diseased palms was analysed to synthesize a medium for culturing the organism but the phytoplasmas failed to grow in various cell-free media and in chick embryos. However, the organisms could be maintained in tissues of diseased palms micropropagated *in vitro* in plant tissue culture media. Remission of symptoms observed in significantly higher numbers of tetracycline-treated palms further confirmed the mycoplasmal etiology of the disease.

RÉSUMÉ

La maladie des racines, non mortelle mais affaiblissant le cocotier, a été signalée depuis plus d'un siècle, maintenant elle affecte 0,41 million d'hectares dans le Kerala, au sud de l'Inde. Elle cause une perte annuelle estimée à environ 968 millions de noix; les palmiers de tous âges sont affectés. Les études sur l'étiologie de la maladie n'ont pas montré le rôle des champignons, des bactéries, des nématodes, des virus, des viroïdes, des protozoaires, des facteurs nutritionnels et physiologiques. Les études ultra-structurales de tissus juvéniles de palmiers malades ont révélé la présence de phytoplasmes (organismes de type mycoplasmes) dans les tubes conducteurs de sève. L'association du phytoplasme à la maladie a été établie d'une manière constante. Les colorations de Dienes et par fluorescence ont été utiles pour la détection des phytoplasmes. Un diagnostic sérologique et un test physiologique ont été développés pour permettre un dépistage précoce de la maladie.

L'inventaire des insectes suspectés comme vecteurs a identifié une cicadelle, *Sophonia greeni*, un derbide, *Proutista moesta* et un tingide, *Stephanitis typica*. Les phytoplasmes ont été détectés dans les glandes salivaires du tingide et du derbide, à la suite d'une phase d'acquisition nutritionnelle et d'incubation sur des arbres malades. Le rôle vecteur du tingide a été établi; la transmission de la maladie par le derbide est en cours d'expérimentation. La transmission expérimentale de la

maladie du cocotier à la pervenche *Catharentlius roseus* est complétée en passant par l'intermédiaire d'un laurier *Cassytha filiformis*. La sève, récoltée sur des palmiers sains et malades, a été analysée pour synthétiser un milieu de culture propice à la multiplication du micro-organisme. Cependant, les phytoplasmes ne se sont pas développés sur divers milieux de cellules libres et dans les embryons de poussin. Toutefois, les organismes ont pu être maintenus dans les tissus de palmiers malades propagés *in vitro*, sur un milieu approprié pour une culture de tissus de plantes. La rémission des symptômes, observée sur un nombre hautement significatif de palmiers traités à la tétracycline, confirme l'étiologie mycoplasmique de la maladie.

INTRODUCTION

This paper highlights the current understanding on the etiology of coconut root (wilt) disease (CRW), a non-lethal but debilitating malady endemic to India. The disease was first noticed around 1874 in three isolated pockets in South Kerala (India) but became more evident after the great floods of 1882 (Butler, 1908; Kunjan Pillai, 1911; Varghese, 1934). A survey in 1984/85 revealed the prevalence of the disease throughout 0.41 million ha in the eight southern districts of Kerala (Central Plantation Crops Research Institute, 1985) in a few isolated pockets in the northern districts of the state (Radha, 1983) and in the bordering districts of Tamil Nadu (Figure 1). The annual loss due to the disease was estimated to be about 968 million nuts; its intensity ranged from 1.5% to 75.6%. Palms of all age groups are affected but infection in the pre-bearing stage delays flowering, affects the normal growth of the palm and contributes to a rapid decline (Ramadasan *et al.*, 1971). About 30% of the CRW-affected palms are affected by a leaf rot disease caused by fungi (Central Plantation Crops Research Institute, 1989a). This adversely affects the yield as the disease progresses (Menon and Nair, 1948, 1951).

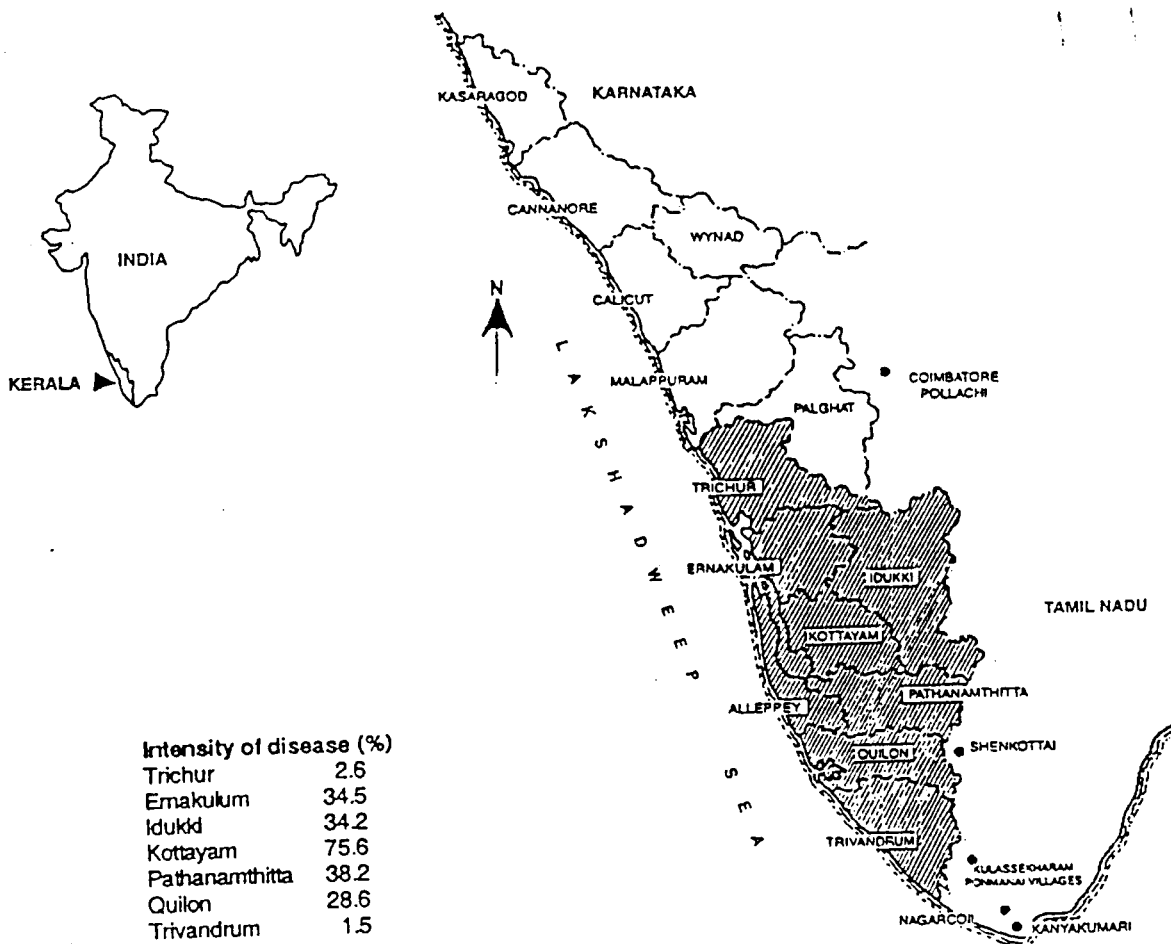


Figure 1 Distribution and intensity of coconut root (wilt) disease in South Kerala, India.

CHARACTERISTICS OF THE DISEASE

Disease spread

The disease occurs in all major soil types but spreads faster in sandy, sandy loam, alluvial and heavy textured soils than in laterites. Higher incidence has been recorded in waterlogged low-lying areas adjacent to rivers and canals (Pillai *et al.*, 1973). The pattern of spread is erratic, occurring in jumps or leaps, characteristic of insect transmission (Pillai *et al.*, 1980) as in Caribbean lethal yellowing (LY) disease (McCoy *et al.*, 1983). But some healthy palms can be observed even in heavily diseased gardens.

Symptoms

An abnormal bending of leaflets termed 'flaccidity' is the most consistent and diagnostic symptom of the disease (Radha and Lal, 1972); foliar yellowing and marginal necrosis are the other common symptoms (Figure 2). Yellowing of foliage is virtually absent in juvenile diseased palms and in seedlings where flaccidity is often the only symptom (Radha and Lal, 1972). An indexing system for quantifying the disease has been developed which gives appropriate weight to the intensity and frequency of occurrence of these three symptoms (George and Radha, 1973; Nambiar and Pillai, 1985). Rotting of roots and the wilting or drooping of leaves are consistently observed in diseased palms and have contributed to the disease being termed 'root (wilt) disease' (Shanta *et al.*, 1960; Mathen *et al.*, 1983). The capacity to regenerate new roots is also drastically affected as the disease progresses (Michael, 1964).

In certain cases, drying up of the spathe and necrosis of spikelets extending from the tip downwards has been noted in tender unopened inflorescences (Menon and Pandalai, 1959;



Figure 2 Root (wilt) diseased palm, Kerala, India.

Maramorosch, 1964). Inflorescence necrosis is also a characteristic symptom of LY disease (McCoy, 1975). A high percentage of pollen produced is either sterile or has low viability (Varkey and Davis, 1960). Lack of ability to produce female flowers and abnormal shedding of female flowers and immature nuts renders the palm unproductive (Varghese, 1934; Menon and Pandalai, 1959). The nuts from diseased palms have a thinner husk and the fibres are definitely weaker and less firm than those from healthy palms (Varghese, 1934). The kernel is thinner and does not dry up into hard brittle copra, remaining soft, rubbery and flexible. The oil content is very much reduced and oil loses its flavour as well (Menon, 1938). Reduction in yield of nuts up to 80% was reported in palms in the advanced stages of disease (Radha *et al.*, 1962).

Anatomical changes observed in the roots of diseased palms are reduction in thickness of the walls of metaxylem elements, resulting in an uneven shape of the xylem component, presence of tyloses, increased colouration and necrotic obliteration of phloem (Govindankutty, 1981). Stomatal dysfunction resulting in excessive water loss (Rajagopal *et al.*, 1986), coupled with poor water absorbing capacity (Central Coconut Research Station, 1970), uptake and transport of water through the trunk (Ramadasan, 1964), and leaching of metabolites due to permeability changes (Central Plantation Crops Research Institute, 1977) are thought to aggravate the disease syndrome.

Diagnosis

Diseased palms are generally identified on the basis of foliar symptoms. A sero-diagnostic test (Solomon *et al.*, 1983) and a physiological test based on stomatal resistance (Rajagopal *et al.*, 1986) have been standardized for detecting the disease. With these tests the disease status can be determined 6–20 months prior to the manifestation of visible symptoms (Rajagopal *et al.*, 1988).

Etiology

Until recently the etiology of this century-old disease remained unresolved; numerous pathogenic agents including fungi, bacteria and nematodes were implicated. Inoculations with these agents singly and in combination failed to induce the disease (Joseph and Lily, 1991; Jayasankar and George, 1991; Sosamma and Koshy, 1991); viruses and viroids were also ruled out (Joseph *et al.*, 1991; Randles and Hatta, 1980).

Physiological and biochemical changes observed in diseased palms were indicative of pathogen-mediated alterations in host metabolism rather than of a physiological disorder (Mathew *et al.*, 1991). Soil sickness as a contributing factor of the disease was also excluded on the basis of field fertilizer trials and mineral analysis of tissue and soil (Cecil and Amma, 1991). Histopathological studies revealed structural changes in the vascular tissues of the diseased palms; disorganization and degeneration of vascular tissues, increased chromogenicity and necrosis were prominent (Govindankutty and Vellaichamy, 1983).

DETECTION OF PHYTOPLASMAS

Electron microscopy

Electron microscopic examination of vascular tissues of juvenile plant parts such as the region below the meristem, immature leaf bases (heart tissues), rachillae of tender inflorescences and root tips of diseased palms revealed the presence of phytoplasmas (mycoplasma-like organisms, MLOs) in sieve tubes (Solomon *et al.*, 1983). These organisms ranged in size from 250 nm to 400 nm, were bound by a trilamellar unit membrane and contained DNA-like strands and peripherally distributed ribosomes. Pleomorphic forms ranging from circular to oval, and occasionally beaded or filamentous, were also observed (Figure 3). The sieve elements which harboured phytoplasmas were immature as they occasionally contained dictyosomes. The organisms were found in increasing numbers in photosynthetic 'sink' regions, suggesting movement within the phloem assimilate stream. Phytoplasmas were unevenly distributed in the

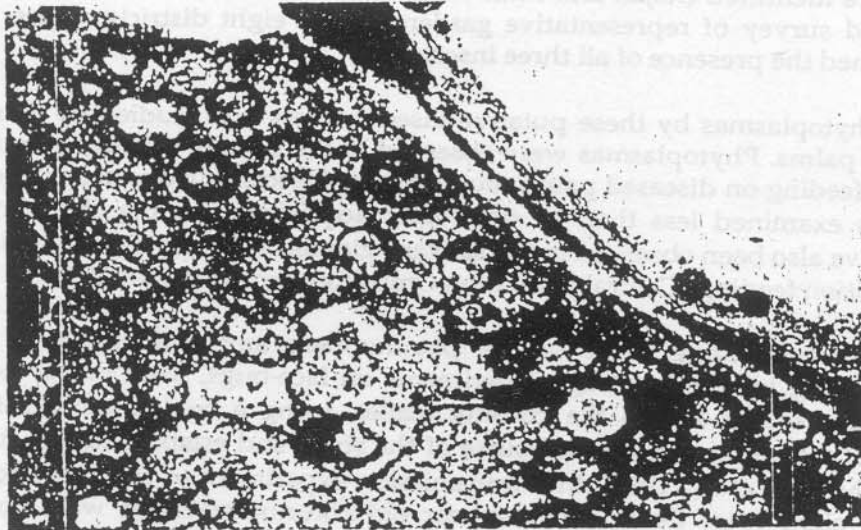


Figure 3 Phytoplasmas in sieve tube of tender rachilla of root (wilt) diseased palm.

sieve tubes: some had none while others had varying concentrations — mature leaf tissues contained few phytoplasmas. Thomas and Norris (1980) also observed fewer organisms in mature organs of LY-affected palms. When only a few occurred, phytoplasmas often assumed a parietal position in the sieve tube. Random distribution and low concentrations are features of LY disease of coconut and lethal decline of palms (Thomas, 1979; Harrison *et al.*, 1994). Phytoplasmas were consistently present in all 75 diseased palms examined of various age groups, intensities of disease and from different locations, but were not detected in an equal number of healthy palms studied.

Light microscopy

Histological staining techniques currently used for visualizing phytoplasmas associated with other diseases have been adopted. Free-hand sections of tender rachillae and roots of diseased palms treated with Dienes' stain exhibited abnormal bluish colouration in sieve tubes. Similarly, free-hand sections of fixed tissues of diseased palms, stained with the fluorochrome DAPI (4'-6-diamidino-2-phenyl indole-2 HCl) and Hoechst 33258, showed fluorescing sites which suggested accumulation of extranuclear DNA, indicating the presence of phytoplasmas (Solomon *et al.*, 1987). Such reactions were not evident in all sieve tubes but were more frequent in junctions of vascular bridges; this was confirmed by ultrastructural studies. Positive staining for Dienes' and fluorochrome was not observed in tissues of healthy palms.

TRANSMISSION STUDIES

Phytoplasmas in general are transmitted by phloem-feeding insects, leafhoppers, planthoppers and, in a few instances, by psyllids. *Myndus crudus*, a cixiid (planthopper), is a vector of LY in the Caribbean region (Howard *et al.*, 1983) and *Myndus adiopodoumeensis* is a suspected vector of lethal yellowing-like disease (LYD) in Africa (Frison *et al.*, 1993). Earlier transmission experiments had implicated a lace-bug, *Stephanitis typica* Distant (Tingidae) — the most common heteropteran found on coconut — as a vector of CRW (Nagaraj and Menon, 1956; Shanta *et al.*, 1964). These studies had presumed a viral etiology and needed reinvestigation in the light of the association of phytoplasmas with the disease. Records of insects on coconut in India did not include any Auchenorrhyncha. Hence an inventory of insects in CRW-affected gardens was made using various traps, sweep-net collections and by direct examination of coconut foliage over a period of two years. A leafhopper, *Sophonia greeni* (Distant), and a planthopper, *Proutista noesta*

(Westwood), were identified (Rajan and Mathen, 1984, 1985) in addition to the lace-bug already reported. A rapid survey of representative gardens in the eight districts where the disease is prevalent confirmed the presence of all three insects.

Acquisition of phytoplasmas by these putative insect vectors was studied by allowing them to feed on diseased palms. Phytoplasmas were observed in the acini of salivary glands of lace-bugs 18–23 days after feeding on diseased palms, but not from insects collected from disease-free areas nor from insects examined less than 18 days after acquisition feeding (Mathen *et al.*, 1987). Phytoplasmas have also been observed in the salivary glands of *P. moesta* examined more than 37 days after acquisition feeding (Central Plantation Crops Research Institute, 1990).

Although the phloem-feeding habit of the planthoppers and leafhoppers is known, such information is lacking for the lace-bug. Experiments on lace-bugs, fixed in the feeding position with the stylet inserted *in situ* in the coconut leaflet, using a cold immobilization technique followed by serial sectioning, traced the course of the stylet and established that it terminated in phloem tissues (Mathen *et al.*, 1988). In a transmission experiment to study the role of the lace-bugs as vectors, eight West Coast Tall two-year old coconut seedlings were obtained from a disease-free area and planted in methyl bromide-fumigated soil held in field tanks of 1.8 x 1.8 x 1.2 m protected with slanting insect-proof cages of 3.7 m³ (Figure 4).

Four of the seedlings were exposed initially to lace-bugs five days after acquisition feeding on diseased palms and these were subsequently supplemented with insects 18 days after acquisition access. To monitor transmission the experimental seedlings were sampled periodically for light and electron microscopy, and for serological tests; visual observation was also made. Positive Dienes' and fluorescent staining, and serological reactions indicated that three of the seedlings exposed to lace-bugs contracted CRW nine months after introduction of insects commenced. Phytoplasmas were observed in ultra-thin sections of root tissues of all four inoculated seedlings between 9 and 27 months after the first exposure. Two of the seedlings showed flaccidity of leaflets — the decisive and diagnostic symptom of the disease — by the 17th month. None of the uninoculated control seedlings either exhibited visual symptoms or had phytoplasmas in the tissues (Mathen *et al.*, 1990). This experiment established conclusively the vector role of the lace-bug; the vector role of the planthopper is also being assessed through a transmission experiment.

Indirect evidence supporting the role of lace bugs as vectors of CRW has also been reported. Larger numbers of lace-bugs were distributed towards the inner leaves of the crown where higher concentrations of phytoplasmas are generally found (Mathen *et al.*, 1969); diseased palms harboured more than four times the number of lace-bugs found on healthy palms (Mathen, 1982),

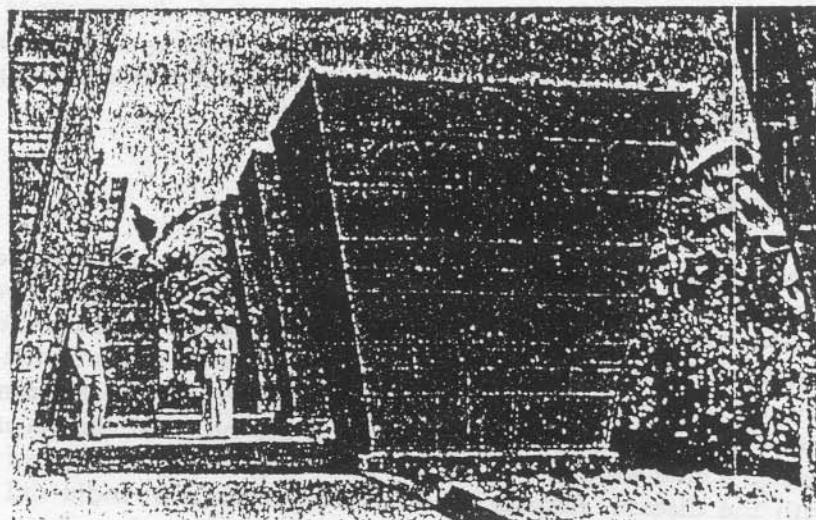


Figure 4 Insect-proof cage for the transmission experiment with lace bugs, CPCRI Regional Station, Kerala.

and there was a direct linear correlation between the number of bugs on a palm and the incidence of disease (Mathen, 1985). Field experiments on the incidence of disease by controlling airborne insects did not yield any conclusive results (Central Plantation Crops Research Institute, 1993). However, reduction in the rate of spread of LY has been achieved by these techniques in Florida (Howard, 1980; Howard and Barrant, 1989).

In addition to the insect transmission studies, experimental transmission with dodder species was also tried. *Cuscuta* species failed to develop intimate haustorial connections with leaf vasculature of coconut. This may be the reason for the failure of Tsai (1983) to transmit LY to coconut using *Cuscuta campestris*. In the present investigation, the disease was transmitted from coconut to periwinkle using the dodder laurel *Cassytha filiformis*. Within three weeks of the establishment of haustorial connections, chlorotic spots appeared in the interveinal areas and vein endings of fully opened leaves of the periwinkle test plants. The presence of phytoplasmas in the diseased source palm, dodder laurel bridge and the recipient periwinkle was also confirmed by electron microscopy (Sasikala *et al.*, 1988).

STUDIES ON CULTURE IN VITRO

Although the consistent association of phytoplasmas with CRW disease and experimental transmission through insect and dodder have been achieved, culturing the organism in cell-free media, and demonstration of its pathogenicity are required to fulfil Koch's postulates. As phytoplasmas are phloem-limited and are thought to have exacting nutritional requirements, an understanding of the physiochemical environment of the phloem may be useful for attempted culture of the organism. Phloem sap with or without supplementary serum or serum components has been found to be a suitable medium for culturing 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.* (1988) improvised a method for collecting phloem sap aseptically in ice-packed vacuum flasks; the sap of diseased palms was found to have higher arginine levels than that from healthy palms. It is surmised that the phytoplasmas present in CRW-diseased palms may be of the non-fermentative type, using arginine through the dihydrolase pathway for their energy requirement (Chempakam and Rajagopal, 1989).

The vascular sap of apparently healthy palms was filter-sterilized and used for *in vitro* culture attempts without supplementation or supplemented with serum. More than 40 different media with various combinations of nutrients and incubation conditions were used for attempted culture of phytoplasmas from tissues of diseased palms, symptomatic periwinkle and infected lace-bugs. Embryonated hen's egg had been reported as a useful *in vivo* model system for cultivation of fastidious organisms like the suckling mouse cataract spiroplasma (Tully *et al.*, 1976). Spiroplasmas from *Sesamum* phyllody could be cultivated in chick embryos (Srinivasan and Solomon, 1992). However, repeated attempts to culture the organisms associated with CRW were unsuccessful in all the media (Central Plantation Crops Research Institute, 1989b). Currently, attempts are being made to maintain and propagate the phytoplasma *in vitro* in tissues of diseased palms micropropagated in plant tissue culture media. Their survival and propagation in culture is being assessed periodically by electron microscopy; which indicates that the organisms can be maintained for 6-8 weeks (Central Plantation Crops Research Institute, 1989). Co-culturing of dodder laurel along with leaf explants from diseased palms and attempted transmission *in vitro* to embryo cultures from healthy palms are in progress.

CHEMOTHERAPY

Since phytoplasmas cannot be cultured *in vitro*, it is recognized that chemotherapy with selected antibiotics can provide circumstantial evidence for mycoplasma etiology of a disease. Antibiotics injected into the plant system should reach the target site quickly and in an unaltered state. A locally made pneumatic pressure injector was used for injecting antibiotics into the trunk (Pillai and Raju, 1985). The uptake, translocation and retention of the antibiotic, oxytetracycline

hydrochloride (OTC) 'Terramycin Tree Formulation' (Pfizer India Ltd.) was studied by bioassay using *Bacillus cereus* subspecies *mycoides* (ATCC 6462). The antibiotic could be detected in the foliage within 24 h of injection and remained detectable at diminishing concentration for more than 12 weeks (Chowdappa *et al.*, 1989).

In a field trial conducted for three years with four concentrations of OTC, single concentrations each of neomycin and penicillin, and a distilled water control, only those palms treated with 3 and 6 g active ingredient of OTC showed remission of symptoms. In contrast, palms injected with penicillin and distilled water deteriorated compared to the pre-treatment condition. Treatment with neomycin had to be discontinued due to severe phytotoxicity (Pillai *et al.*, 1991). The remission of symptoms observed in OTC-treated palms is further evidence of the mycoplasmal etiology of this disease.

CONCLUSION

Since no effective control measures are available for combating the disease, the only alternative is to breed for disease resistance, manage the diseased gardens with the recommended package of practices and prevent spread of the disease to newer areas by removal of diseased palms from isolated pockets where incidence is still sparse. Future work will concentrate on detection and characterization of CRW phytoplasmas using polymerase chain reaction (PCR) and use of restriction fragment length polymorphism (RFLP) to examine the relationships to LYD which occur in Caribbean and African countries.

REFERENCES

- BUTLER, E.J. (1908) Report on coconut palm disease in Travancore. *Agricultural Research Institute Bulletin* No. 9. Pusa, India: Agricultural Research Institute.
- CECIL, S.R. and AMMA, P.G.K. (1991) Soils and nutrition. pp. 45–52. In: Coconut Root (Wilt) Disease. *Monograph Series* No. 3. NAIR, M.K., NAMBLAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL COCONUT RESEARCH STATION (1970) *Annual Report for 1968*. Kayangulam, India: Central Coconut Research Station.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1977) *Annual Report for 1976*. Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1985) *Coconut Root (Wilt) Disease Intensity, Production Loss and Future Strategy*. Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1989a) Coconut root (wilt) disease — a discourse. *Technical Bulletin* No. 19. Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1989b) *Annual Report for 1988*. Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1990) *Annual Report for 1989–90*. Kasaragod, India: Central Plantation Crops Research Institute.
- CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (1993) *Annual Report for 1992–93*. Kasaragod, India: Central Plantation Crops Research Institute.

CHEMPAKAM, B. and RAJAGOPAL, V. (1989) Biochemical composition of vascular sap from the inflorescence of apparently healthy and root (wilt)-diseased coconut palms. *Journal of Plantation Crops*, 16 (Supplement): 41-46.

CHOWDAPPA, P., PILLAI, N.G., GUNASEKARAN, M. and SOLOMON, J.J. (1989) Distribution and retention of oxytetracycline hydrochloride injected into coconut palms affected by root (wilt) disease. *Journal of Plantation Crops*, 17: 108-113.

EDEN-GREEN, S.J. and WATERS, H. (1982) Collection and properties of phloem sap from healthy and lethal yellowing diseased coconut palms in Jamaica. *Phytopathology*, 72: 667-672.

FRISON, E.A., PUTTER, C.A.J. and DIEKMANN, G. (eds) (1993) *FAO/IBPGR Technical Guidelines for the Safe Movement of Coconut Germplasm*. Rome: Food and Agricultural Organization of the United Nations/International Board for Plant Genetic Resources.

GEORGE, M.V. and RADHA, K. (1973) Computation of disease index of root (wilt) disease of coconut. *Indian Journal of Agricultural Science*, 43: 366-370.

GOVINDANKUTTY, M.P. (1981) Histopathological studies. pp. 49-54. In: *Review of Research on Coconut Root (Wilt) Disease*. Kayangulam, India: Central Plantation Crops Research Institute, Regional Station. [Mimeo]

GOVINDANKUTTY, M.P. and VELLAICHAMY, K. (1983) Histopathology of coconut palm affected with root (wilt) disease. pp. 421-425. In: *Coconut Research and Development*. NAYAR, N.M (ed.). New Delhi: Wiley Eastern Ltd.

HARRISON, N.A., RICHARDSON, P.A., KRAMER, J.B. and TSAI, J.H. (1994) Detection of the mycoplasma-like organism associated with lethal yellowing disease of palms in Florida by polymerase chain reaction. *Plant Pathology*, 43: 998-1008.

HOWARD, F.W. (1980) Population densities of *Myndus crudus* Van Duzee (Homoptera: Cixiidae) in relation to coconut lethal yellowing in Florida. *Principes*, 24: 174-178.

HOWARD, F.W. and BARRANT, C.I. (1989) Question and answers about lethal yellowing disease. *Principes*, 33: 163-171.

HOWARD, F.W., NORRIS, R.C. and THOMAS, D.L. (1983) Evidence of transmission of palm lethal yellowing agent by a planthopper *Myndus crudus* (Homoptera: Cixiidae). *Tropical Agriculture (Trinidad)*, 60: 168-171.

JAYASANKAR, N.P. and GEORGE, M. (1991) Etiology — B. Bacteria. pp. 17-18. In: *Coconut Root (Wilt) Disease*. *Monograph Series No. 3*. NAIR, M.K., NAMBIAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.

JOSEPH, T. and LILLY, V.G. (1991) Etiology — A. Fungi. pp. 13-15. In: *Coconut Root (Wilt) Disease*. *Monograph Series No. 3*. NAIR, M.K., NAMBIAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.

JOSEPH, T., PILLAI, N.G. and SOLOMON, J.J. (1991) Etiology — D. Virus. pp. 27-30. In: *Coconut Root (Wilt) Disease*. *Monograph Series No. 3*. NAIR, M.K., NAMBIAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.

KUNJAN PILLAI, N. (1911) *Naleekeram*. Quilon, Travancore, India: Vidyabhivardhini Press.

MARAMOROSCH, K. (1964) *A Survey of Coconut Diseases of Unknown Etiology*. Rome: Food and Agricultural Organization of the United Nations.

- MATHEN, K. (1985) Lace-bug abundance in root (wilt) disease affected coconut palms — cause or effect? *Journal of Plantation Crops*, 13: 56–59.
- MATHEN, K., MATHEW, J. and KURIAN, C. (1969) Pattern of distribution of *Stephanitis typica* (Distant) (Heteroptera: Tingidae), a pest of coconut palm on its host. *Indian Journal of Agricultural Science*, 39: 57–61.
- MATHEN, K., NAIR, C.P.R., GUNASEKARAN, M., GOVINDANKUTTY, M.P. and SOLOMON, J.J. (1988) Stylet course of lace-bug *Stephanitis typica* (Distant) in coconut leaf. *Proceedings of the Indian Academy of Science*, 97: 539–544.
- MATHEN, K., PILLAI, N.G. and RADHA, K. (1983) Terminology of coconut root (wilt) disease, pp. 342–346. In: *Coconut Research and Development*. NAYAR, N.M. (ed.). New Delhi: Wiley Eastern Ltd.
- MATHEN, K., RAJAN, P., NAIR, C.P.R., SASIKALA, M., GUNASEKARAN, M., GOVINDANKUTTY, M.P. and SOLOMON, J.J. (1990) Transmission of root (wilt) disease to coconut seedlings through *Stephanitis typica* (Distant) (Heteroptera: Tingidae). *Tropical Agriculture (Trinidad)*, 67: 69–73.
- MATHEN, K., SOLOMON, J.J., RAJAN, P. and GEETHA, L. (1987) Electron microscopic evidence on the role of *Stephanitis typica* (Distant) as vector of coconut root (wilt) disease. *Current Science*, 56: 1339–1341.
- MATHEW, C., RAJAGOPAL, V., VARKEY, T. and JOSEPH, K.V. (1991) Physiology and biochemistry. pp. 53–59. In: *Coconut Root (Wilt) Disease. Monograph Series No. 3*. NAIR, M.K., NAMBIAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.
- MCCOY, R.E. (1975) Effect of oxytetracycline dose and stage of disease development on remission of lethal yellowing in coconut palm. *Plant Disease Reporter*, 59: 717–720.
- MCCOY, R.E. (1976) Plant phloem sap: a potential mycoplasma growth medium. *Proceedings of the Society for General Microbiology*, 3: 155.
- MCCOY, R.E. (1977) Growth of mycoplasmas in phloem sap from lethal yellowing resistant Malayan dwarf coconut palm. *Proceedings of the American Phytopathological Society*, 4: 108.
- MCCOY, R.E. 1978. Growth of the plant parasitic protozoan *Phytomonas davidi* in mycoplasma media and in phloem sap. *Phytopathology News*, 12: 217.
- MCCOY, R.E., HOWARD, F.W., TSAI, J.H., DONSELMAN, H.M., THOMAS, D.L., BASHAM, H.G., ATILANO, R.A., ESKAFI, F.M., BRITT, L. and COLLINS, M.E. (1983) Lethal Yellowing of Palms. *University of Florida Agricultural Experiment Stations Bulletin* 834. Gainesville, Florida: University of Florida.
- MENON, K.P.V. (1938) *Report on the Scheme of Investigations on the Diseases of Coconuts in South India, 1937*. New Delhi: Government of India Press.
- MENON, K.P.V. and NAIR, V.K. (1948) The leaf rot disease of coconut in Travancore and Cochin. *Indian Coconut Journal*, 1: 33–39.
- MENON, K.P.V. and NAIR, V.K. (1951) Scheme for the investigations of the root and leaf diseases of the coconut palm in South India. Final report of work from 8 March 1937 to 31 March 1948. *Indian Coconut Journal*, 5: 3–19.

MENON, K.P.V. and PANDALAI, K.M. (1959) *The Coconut Palm – A Monograph*. Ernakulam, India: Indian Central Coconut Committee.

MICHAEL, K.J. (1964) Studies on the root system of the coconut palm. *Indian Coconut Journal*, 9: 85–92.

NAGARAJ, A.N. and MENON, K.P.V. (1956) Note on the etiology of the wilt (root) disease of coconut palms in Travancore – Cochin. *Indian Coconut Journal*, 9: 161–165.

NAMBIAR, P.T.N. and PILLAI, N.G. (1985) A simplified method of indexing root (wilt) affected coconut palms. *Journal of Plantation Crops*, 13: 35–37.

PILLAI, N.G., CHOWDAPPA, P., SOLOMON, J.J. and MATHEW, J. (1991) Remission of symptoms of root (wilt) disease of coconut injected with oxytetracycline-HCl. *Journal of Plantation Crops*, 19: 14–20.

PILLAI, N.G., LAL, S.B. and SHANTA, P. (1973) Distribution and intensity of root (wilt) disease of coconut in Kerala. *Journal of Plantation Crops*, 1 (Supplement): 107–112.

PILLAI, N.G. and RAJU, C.A. (1985) A simple pressure injection device for application of antibiotics on coconut palm. *Journal of Plantation Crops*, 13: 151–154.

PILLAI, N.G., SASIKALA, M. and MATHEN, K. (1980) Epidemiology of coconut root (wilt) disease. *Journal of Plantation Crops*, 8: 90–97.

RADHA, K. (1983) Occurrence of coconut root (wilt) disease in Northern Kerala and possible approach to control the disease. *Indian Coconut Journal*, 13: 28–31.

RADHA, K. and LAL, S.B. (1972) Diagnostic symptom of root (wilt) disease of coconut. *Indian Journal of Agricultural Science*, 42: 410–413.

RADHA, K., SAHASRANAMAN, K.M. and MENON, K.P.V. (1962) A note on the yield of coconut in relation to rainfall and leaf rot and root (wilt) disease. *Indian Coconut Journal*, 16: 3–11.

RAJAGOPAL, V., CHEMPAKAM, B., AMMA, P.G.K. and CECIL, S.R. (1988) Method of collection and properties of vascular sap from the inflorescences of healthy and root (wilt) diseased coconut palm. *Plant Physiology and Biochemistry*, 15: 308–312.

RAJAGOPAL, V., PATIL, K.D. and AMMA, B.S.K. (1986) Abnormal stomatal opening in coconut palms affected with root (wilt) disease. *Journal of Experimental Botany*, 37: 1398–1405.

RAJAGOPAL, V., SASIKALA, M., AMMA, B.S.K., CHEMPAKAM, B. and RAWTHER, T.S.S. (1988) Early diagnostic techniques on the root (wilt) disease of coconut in India. *Philippine Journal of Coconut Studies*, 13: 31–35.

RAJAN, P. and MATHEN, K. (1984) *Sophonia greenii* (Distant) (Nirvanidae: Jassoidea) on leaves of coconut palm, *Cocos nucifera* L. *Journal of Plantation Crops*, 12: 178–179.

RAJAN, P. and MATHEN, K. (1985) *Proutista moesta* (Westwood) and other additions to insect fauna on coconut palm. *Journal of Plantation Crops*, 13: 135–136.

RAMADASAN, A. (1964) Physiology of wilt disease in coconut palms. pp. 257–272. In: *Proceedings of Second Session of the FAO Working Party on Coconut Production, Protection and Processing, Colombo*. Rome: Food and Agricultural Organization of the United Nations.

- RAMADASAN, A., SHANTA, P. and LAL, S.B. (1971) Resistance or susceptibility, age of bearing and yield of coconut in young palms in relation to the development of root (wilt) disease. *Indian Journal Agricultural Science*, 41: 1107–1109.
- RANDLES, J.W. and HATTA, T. (1980) Etiological studies on virus-like diseases of coconut and cardamom in India. In: *Biennial Report of the Waite Agricultural Research Institute, 1978–79*. Melbourne, Australia: Waite Agricultural Research Institute.
- SASIKALA, M., MATHEN, K., GOVINDANKUTTY, M.P., SOLOMON, J.J. and GEETHA, L. (1988) Transmission of mycoplasma-like organisms from *Cocos nucifera* with root (wilt) to *Catharanthus roseus* by *Cassyltha filiformis*. *Netherlands Journal of Plant Pathology*, 94: 191–194.
- SHANTA, P., JOSEPH, T. and LAL, S.B. (1964) Transmission of root (wilt) disease of coconut. *Indian Coconut Journal*, 18: 25–28.
- SHANTA, P., MENON, K.P.V. and PILAI, K.P. (1960) Aetiology of the root (wilt) disease: Investigation on its virological nature. *Indian Coconut Journal*, 13: 56–66.
- SOLOMON, J.J., GOVINDANKUTTY, M.P. and MATHEN, K. (1987) Detection of mycoplasma-like organisms in root (wilt) disease affected coconut and its putative insect vector. Paper read at: *Third Regional Workshop on Plant Mycoplasma, New Delhi, 24–28 February*. [Unpublished]
- SOLOMON, J.J., GOVINDANKUTTY, M.P. and NIENHAUS, F. (1983) Association of mycoplasma-like organisms with coconut root (wilt) disease in India. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 90: 295–297.
- SOLOMON, J.J., SASIKALA, M. and SHANTA, P. (1983) A serological test for the detection of root (wilt) disease of coconut, pp. 401–405. In: *Coconut Research and Development*. NAYAR, N.M. (ed.). New Delhi: Wiley Eastern Ltd.
- SOSAMMA, V.K. and KOSHY, P.K. (1991) Etiology — C. Nematodes. pp. 19–26. In: *Coconut Root (wilt) Disease*. *Monograph Series No. 3*. NAIR, M.K., NAMBIAR, K.K.N., KOSHY, P.K. and JAYASANKAR, N.P. (eds). Kasaragod, India: Central Plantation Crops Research Institute.
- SRINIVASAN, N. and SOLOMON, J.J. (1992) Cultivation of spiroplasma from *Sesamum* affected by phyllody in chick embryos. *Journal of Current Bioscience*, 9: 97–102.
- THOMAS, D.L. (1979) Mycoplasma-like bodies associated with lethal declines of palms in Florida. *Phytopathology*, 69: 928–934.
- THOMAS, D.L. and NORRIS, R.C. (1980) The use of electron microscopy for lethal yellowing diagnosis. *Proceedings of the Florida State Horticultural Society*, 93: 196–199.
- TSAI, J.H. (1983) Attempts to transmit lethal yellowing of coconut palms by suspected vectors. pp. 415–420. In: *Coconut Research and Development*. NAYAR, N.M. (ed.). New Delhi: Wiley Eastern Ltd.
- TULLY, J.G., WHITCOMB, R.F., WILLIAMSON, D.L. and CLARK, H.F. (1976) Suckling mouse cataract agent is a helical wall-free prokaryote. *Nature (London)*, 259: 117–120.
- VARGHESE, M.K. (1934) *Disease of Coconut Palm*. Travancore, India: Department of Agriculture and Fisheries.
- VARKEY, T. and DAVIS, T.A. (1960) Studies on coconut pollen with reference to the leaf and root (wilt) disease. *Indian Coconut Journal*, 14: 1–7.