Transmission of root (wilt) disease to coconut seedlings through *Stephanitis typica* (Distant) (Heteroptera:Tingidae)

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Two-year-old West Coast Tall coconut seedlings obtained from a disease-free area were planted in methyl bromide-fumigated loamy sand collected from paddy field, held in field tanks and protected inside netted field cages. The seedlings were regularly inoculated with infective lace bugs, *Stephanitis typica*. Spear-leaf tissues of three of the four experimental seedlings gave strong positive serological reactions, indicating infection, nine months after the first inoculation. Root tissues treated with Dienes's stain, DAPI and Hoechst 33258 fluorochromes indicated mycoplasma-like organisms (MLO) infection in the phloem. Serological and stain reactions in the fourth seedling were feeble at that time. Electron microscope examination of ultra thin sections of root apexes of all the seedlings revealed the presence of mycoplasma-like organisms in the sieve tubes. Of the four seedlings to which MLOs were transmitted by the tingids, two developed flaccidity of leaflets, the diagnostic and decisive symptom of the disease, eight months after infection was indicated by serodiagnosis. By the time the seedlings were visually identified as manifesting the symptom of the disease, each seedling had received more than 1000 lace bugs with five days' acquisition feeding on diseased palms known to harbour MLOs and about 1130 lace bugs with five days' acquisition and thirteen days' incubation. Control plants remained free of the organism and the symptoms of the disease.

Keywords: Coconut root wilt; MLO; Lace bug vector; *Stephanitis typica*; Fluorescence; Electron microscopy

A non-lethal but debilitating disease of the coconut palm, today known as root (wilt), was reported from three separate areas of Kerala State, India, about a century ago. During the past one hundred years, the disease has spread about 200 km north and south of the places of first occurrence. It is limited by natural boundaries of the Arabian Sea on the west and the Western Ghats on the east. Today it is prevalent in different degrees of intensity over a contiguous area of 410 000 ha, causing a loss of 968 million nuts in 1986 (Anonymous, 1986 a).

A virus aetiology of the disease was proposed by Nagaraj *et al.* (1954). Nagaraj and Menon (1956) attributed a vector role to lace bugs on the basis of their being the major group of insect visitors of coconut foliage. Experiments carried out by them, Shanta *et al.* (1960), Shanta *et al.* (1964) and Joseph *et al.* (1972) provided evidence on the transmission of the disease by *Stephanitis typica*. However, when Solomon *et al.* (1983) reported an association of mycoplasma-like organisms in the tissues of diseased palms, but not of healthy palms, MLOs were included in the list of probable causal agents of the disease and the role of a tingid bug as a possible vector of MLOs was reconsidered. Examination of the insect under the electron microscope revealed the presence of MLOs in the salivary gland and brain of lace bugs allowed to feed *in vivo* for five days on leaflets of diseased palms known to harbour MLOs (through electron microscopy) and incubated 13–18 days (Mathen *et al.*, 1987). Mathen (1982) had reported four times as many lace bugs infesting diseased palms as the healthy. Mathen (1985) brought out a positive linear correlation between the tingid colonization level and fresh incidence of the disease. Thus, the lace bug emerged strongly as a putative vector of the disease. An experiment was therefore carried out to see if coconut seedlings would contract the disease when inoculated with infective lace bugs.

Materials and methods

Plant material

Two-year-old West Coast Tall coconut seedlings, enclosed in polythene bags, were brought to the research station from Kidu (Karnataka State), a disease-free area. Their boles were dipped in 1000 ppm phorate slurry in water for 30 min in order to destroy any infestation of roots by burrowing nematodes. 16 seedlings of nearly uniform growth and other morphological characters were selected from the lot of 30 and planted 25 June 1985; eight (four for insect liberation and four as control) were set in field tanks (1.8 × 1.8 × 1.2 m) holding loamy-sand soil from paddy fields, fumigated on 14 June 1985 with methyl bromide at 1 kg h 4 m^{-3}; four were placed in similar tanks filled with unfumigated soil from a coconut garden of Kayangulam, a disease-prevalent area; and four were planted in the open. All 16 seedlings were sprayed with a mixture of 0.03% dithane M-45 and 0.05% malathion on 26 June 1985 to take care of any initial fungal infection or insect infestation.

Field cages

Each of the 12 field tanks was already provided with

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a 3.7 m high slanting cage (Figure 1) supported on iron frames and netted with galvanized iron gauze, 40 mesh linear inch^{-1}. Entry to the cage was through a hinged door (1.8 × 0.8 m), guarded by a netted portable ante-room. Repairs to the nets were attended to periodically and spray painting was carried out before and after the rainy seasons, during which the experimental seedlings inside were protected by covering with polythene sheets.

Insect inoculation

Adult lace bugs, collected from coconut palms in the field at Kayangulam, were allowed to feed for five days on leaflets of diseased palms known to harbour MLOs (diagnosed by electron microscopy) by caging batches of 25 insects in long, loose muslin cloth bags. This was to ensure that they acquired the MLOs as the insect collections were mainly from coconut seedlings free of disease. The survivors were used in two different ways for inoculation. In the first, they were immediately liberated into the four cages in equal numbers, their survival being ensured by counting them after a fortnight. The first inoculation, given on 18 September 1985, was supplemented by daily liberations to the end of June 1986. In the second, the recaptured insects were maintained for another 13 days on coconut leaflets to ensure infectivity after incubation. Inoculation with such insects started in July 1986 and was carried through the two quarters of 1986 and first quarter of 1987. In the second and third quarters of 1987, we reverted to inoculation with insects subjected only to acquisition. In the last quarter of 1987, liberation of insects was restricted to seedlings in the two cages in which symptom had not appeared (Table 1).

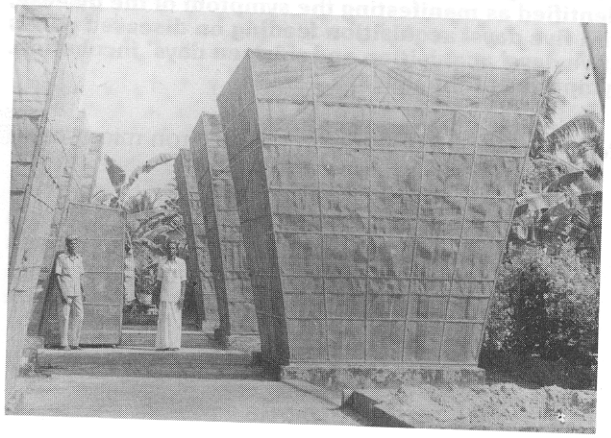

Figure 1 Netted cages erected over field tanks

Methods for testing infection

Leaflets from spear leaves of seedlings were serologically examined according to the method described by Solomon *et al.* (1976). Fresh root-tips were subjected to light and electron microscope examination as detailed by Solomon *et al.* (1983), Solomon *et al.* (1987).

Results

Table 1 gives details of the number of lace bugs inoculated, compiled for the different quarters of 1985, 1986 and 1987. The sample of plant tissue drawn in June 1986, nine months after the initial inoculation and subjected to serological and light microscopic examination, yielded strong positive reactions in three seedlings and a feeble reaction in the fourth. In the agar gel double-diffusion test, a crisp precipitin line was observed midway between the disease antigen and the root (wilt) antiserum reservoirs; no precipitin line was formed against the sample from the control. The inoculated plants had contracted the disease. With light microscopy, sections of root apexes treated with Dienes' stain developed a bluish colouration in the sieve tubes and induced abnormal fluorescence with DAPI and Hoechst 33258 fluorochromes, indicating MLO infection in the phloem. The tissues fixed for electron microscopy were therefore immediately processed and examined. MLOs were noticed in the sample of root apexes (Figure 2) drawn in June 1986 from one seedling, establishing that transmission of the MLOs

Table 1 Number of lace bugs inoculated on experimental coconut seedlings in field cages numbered 3, 4, 9 and 10

Date	5 days' acquisition					5 days' acquisition plus 13 days' incubation				
	3	4	9	10	Total	3	4	9	10	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					

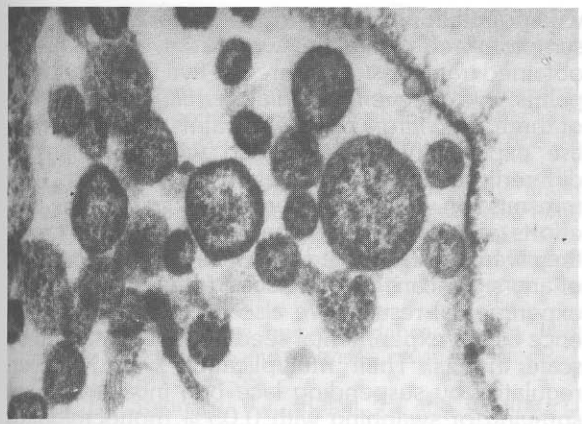

Figure 2 MLOs in root apex of coconut seedling inoculated with infective lace bugs

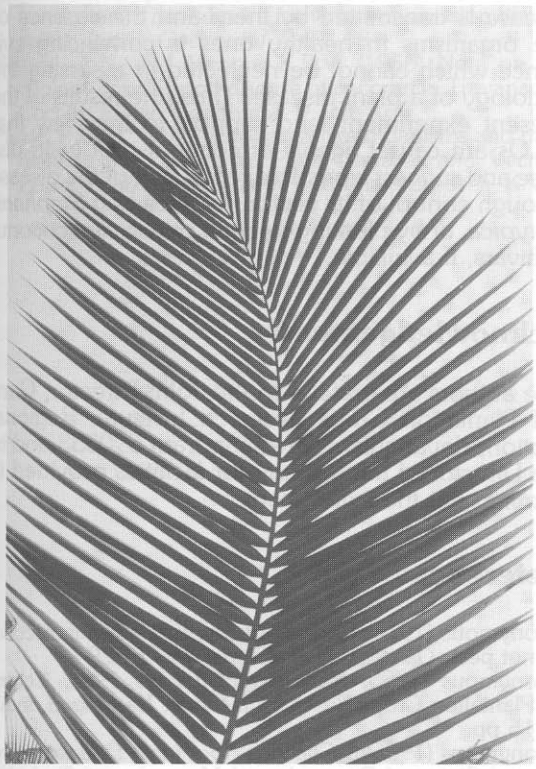

Figure 3 Normal leaflets of a coconut seedling

had taken place. The seedling had by then received 1013 lace bugs with five days' acquisition feed on diseased palms (Table 1). Electron microscopy of samples collected in September 1986 from two other seedlings showed the organisms. In the fourth seedling, the organisms were seen in the root sampled as late as 30.12.1987; by November 1987 it had received a total of 2108 lace bugs with five days' acquisition and 1129 lace bugs with five days' acquisition plus 13 days' incubation (Table 1).

From July 1986 the seedlings were carefully watched for expression of visual symptoms. Flaccidity of leaflets was noticed in its initial stage in two seedlings in February 1987 (17 months after the first inoculation), becoming evident by June 1987 (21 months after the first inoculation) (Figures 3 and 4). By then the seedlings had received 1143 and 1130 lace bugs with five days' acquisition plus 13 days' incubation in addition to 1015 and 1021 lace bugs,

Figure 4 Leaflets showing flaccidity, the decisive symptom of root (wilt) disease, consequent on inoculation with infective lace bugs

respectively, with five days' acquisition only till the end of June 1986. The seedlings became cage-bound and were therefore uprooted in February 1988. Rotting of roots, another symptom of the disease reported under field conditions but not expected as the seedlings were growing in soil initially fumigated with methyl bromide, was not significant. Control seedlings did not give serological and light microscope reactions nor did they present MLOs or develop flaccidity of leaflets.

Discussion

Leach (1940) laid down four requirements to provide adequate proof of insect transmission of a plant disease. They are (i) demonstration of a close though not necessarily constant association of the insect with diseased plants; (ii) regular visits to healthy plants by the insect; (iii) presence of the microorganism associated with the diseased plant in the insect following visits to diseased plant; and (iv) production of disease in experimental plants under controlled conditions as a result of inoculating with infective insects, with sufficient checks. *S. typica*, reported as a minor pest of coconut foliage (Anonymous, 1915), is present all over the State as the most abundant insect visitor on the palms throughout the year (Mathen *et al.*, 1968; Mathen, 1982). The disease does not occur independently of the tingids; in representative localities of the eight districts of the disease-prevalent area, there was no garden free of lace bugs which were also observed on palms of isolated disease-prevalent gardens beyond the contiguous area of disease incidence. Healthy palms of the disease-free areas and apparently healthy (symptomless) palms of diseased tracts also har-

boured colonies of the bug. Mathen *et al.* (1987) reported the presence of MLOs in the salivary glands of lace bugs submitted to five days' acquisition and 13–18 days' incubation and their absence in lace bugs collected from disease-free areas. The diseased condition of the experimental plants inoculated with infective lace bugs was demonstrated through positive results of serological and histochemical stain reactions. The organisms have been observed under electron microscopy in the sieve tubes of roots. Flaccidity of leaflets, the diagnostic symptom of the disease (Radha and Lal, 1972), was developed in the insect-inoculated seedlings but was absent in uninoculated control plants. The norms of acceptable insect transmission have thus been fulfilled.

The experiments conducted by Nagaraj and Menon (1956) and Shanta *et al.* (1960) had the limitation that inoculations were carried out on palms in the open, so that control palms were also prone to natural infection. Positive results of transmission were assessed on a higher percentage incidence of disease in treated palms than in the control. Shanta *et al.* (1964) had the advantage of carrying out the experiment in an insect-proof house with coconut seedlings planted in steam-sterilized soil in cement tubs. They obtained one seedling diseased out of the six inoculated with lace bugs which had been given an acquisition feed for 24 h. The present experiment was conducted in large field tanks covered by field cages to hold test insects, with a better understanding of the probable causal organism and improved technique of insect inoculation. Whereas Shanta *et al.* (1964) obtained the symptom of flaccidity 42 months after the first inoculation, in the present study, flaccidity of leaflets was manifested in about 17 months after the first liberation of lace bugs in two of the four seedlings to which MLOs were transferred. According to Radha and Lal (1972), flaccidity is the sole symptom of disease expression in seedlings and young palms; other symptoms such as yellowing and marginal necrosis of leaflets occur in adult palms.

MLO diseases in plants are generally transmitted by leaf hoppers and plant hoppers. The systematic position of lace bugs (Tingidae) outside these groups was the main consideration for conducting the present experiment of a repetitive nature. The result has confirmed the role of lace bugs in transmitting coconut root (wilt) disease and the associated MLOs. Support from literature in this regard is available from two instances of Piesmidae, taxonomically close to Tingidae, reported to be vectors of phloem-bound MLOs and RLOs – sugar beet savoy disease in North America, earlier considered to be a virus disease (Coons *et al.*, 1958) but now reckoned as of MLO etiology (Harris, 1979) transmitted by *Piesma cinereum* (Say); and sugar beet latent rosette disease in the German Democratic Republic caused by RLO, vectored by *Piesma quadratum* (Fieb.) (Proeseler, 1980). A study of the feeding probe of *S. typica* into the coconut leaf demonstrated the termination of the stylet in phloem tissue (Anonymous, 1986 b), thereby showing the ability of the bug to acquire the phloem-seated MLOs.

MLO-associated lethal yellowing of coconut in Florida was transmitted by the plant hopper *Myndus crudus* van Duzee to several palms including coconut (Howard *et al.*, 1983, 1984). The number of insects liberated was very high, probably because wild populations which included plant hoppers from symptom-free palms also were used. In 1983, they obtained transmission in three of five *Cocos nucifera* palms, five of seven *Veitchia merrillii* palms and two of three *Pritchardia thurstonii* palms. In 1984, when the experiment was repeated with 98 palms of different genera and species, they obtained 8.16% transmission. They reported that in spite of the best efforts at prompt maintenance of the field cages, they were contaminated with insect infestations, not of any significance whatsoever, to interfere with the experimental results. We also had a similar experience as the experimental seedlings were infested by scale insects. Their multiplication was, however, regulated by suspending lace bug inoculation and spraying or swabbing with 0.05% monocrotophos or endosulfan.

Ploaie (1981) remarked that the presence of MLOs in the insect vectors and the plants to which disease is transmitted by them and the absence of the organisms in healthy ones is convincing evidence which cannot be neglected in assessing the aetiology of a plant disease. Thus, the results of the present experiment lend support to the view that MLOs are causal agents of coconut root (wilt) disease and suggest a possible regulation of the disease through control of its vector, the lace bug *Stephanitis typica*, or by planting tolerant or resistant coconut cultivars, if available.

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References

Anonymous (1915) *Cadamustus typicus* – a minor coconut pest, *Gardens' Bull. (Singapore)* **1** 329

Anonymous (1985) *Research Highlights 1984*, Central Plantation Crops Research Institute, Kasaragod, India, 15 pp

Anonymous (1986 a) *Coconut Root (wilt) Disease – Present Status of Research and Management*, Technical Bulletin **14**, Central Plantation Crops Research Institute, Kasaragod, India, 10 pp

Anonymous (1986 b) *Research Highlights 1985*, Central Plantation Crops Research Institute, Kasaragod, India, 15 pp

Coons, G.H., Stewart, D., Bockstahler, H.W. and Schneider, C.L. (1958) Incidence of savoy disease in relation to the variety of sugar beets and to the proximity of wintering habitat of the vector *Piesma cinerea*, *Plant Dis. Reptr.* **42** 502–511

Harris, K.F. (1979) Leaf-hoppers and aphids as biological vectors: vector-virus relationship, in: *Leaf-hopper Vectors and Plant Disease Agents* (eds. Maramorosch, K. and Harris, K.F.), New York, Academic Press, 654 pp

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

Howard, F.W., Williams, D.S. and Norris, R.C. (1984) Insect transmission of lethal yellowing to young palms, *Int. J. Entomol.* **26** 331–338

Joseph, T., Shanta, P. and Lal, S.B. (1972) Role of *Stephanitis typicus* Dist. in the spread of coconut root (wilt) pathogen, *Indian J. Agric. Sci.* **42** 414–417

Leach, J.G. (1940) *Insect Transmission of Plant Diseases*, New York and London, McGraw-Hill Book Co. Inc., 615 pp

Mathen, K., Mathew, J. and Kurian, C. (1968) Seasonal abundance of populations of *Stephanitis typicus* Dist. in the field of coconut palm, *Indian J. Agric. Sci.* **38** 644–653

Mathen, K. (1982) Comparative abundance of the tingid vector *Stephanitis typica* (Dist.) in the root (wilt) affected and healthy coconut palms in Kerala, *Proceedings All India Symposium on Vectors and Vector-borne Diseases*, University of Kerala, Trivandrum, India, 227 pp

Mathen, K. (1985) Lace bug abundance in root (wilt) disease affected coconut palms – cause or effect? *J. Plant. Crops* **13** 56–59

Mathen, K., Solomon, J.J., Rajan, P. and Geetha, L. (1987) Electron microscopic evidence on the role of *Stephanitis typica* (Dist.) as vector of coconut root (wilt) disease, *Curr. Sci.* **56** 1239–1241

Nagaraj, A.N., Davis, T.A. and Menon, K.P.V. (1954) Sap transfusion, a new device for virus transmission trials in palms, *Indian Coconut J.* **7** 91–98

Nagaraj, A.N. and Menon, K.P.V. (1956) Note on the etiology of wilt (root) disease of coconut in Travancore-Cochin, *Indian Coconut J.* **9** 161–165

Ploaie, P.G. (1981) Mycoplasma-like organisms and plant diseases in Europe, in: *Plant Diseases and Vectors: Ecology and Epidemiology* (eds. Maramorosch, K. and Harris, K.F.), New York, Academic Press, 368 pp

Proeseler, G. (1980) Piesmids, in: *Vectors of Plant Pathogens* (eds. Harris, K.F. and Maramorosch, K.), New York, Academic Press, 467 pp

Radha, K. and Lal, S.B. (1972) Diagnostic symptoms of root (wilt) disease of coconut, *Indian J. Agric. Sci.* **42** 410–413

Shanta, P., Menon, K.P.V. and Patchu Pillai, K. (1960) Aetiology of the (wilt) root disease: Investigations on its virological nature, *Indian Coconut J.* **13** 55–66

Shanta, P., Joseph, T. and Lal, S.B. (1964) Transmission of root (wilt) disease of coconut, *Indian Coconut J.* **18** 25–28

Solomon, J.J., Sasikala, M. and Shanta, P. (1976) A serological test for the detection of root (wilt) disease of coconut, *Proceedings of International Symposium on Coconut Research and Development* (ed. Nayar, N.M.), Indian Society for Plantation Crops, Kasaragod, India, 518 pp

Solomon, J.J., Govindankutty, M.P. and Nienhaus, F. (1983) Association of mycoplasma-like organisms with the coconut root (wilt) disease in India, *Z. PflKrankh. Pflschutz* **90** 295–297

Solomon, J.J., Govindankutty, M.P. and Mathen, K. (1987) Detection of MLOs in root (wilt) disease-affected coconut and its putative vector, *Proceedings of III Regional Workshop on Plant Mycoplasma* 24–28 February, New Delhi (in press)