4

DIAGNOSIS

M. SASIKALA, B.S.K. AMMA and V. RAJAGOPAL

The coconut root (wilt) disease is identified on the basis of visual symptoms. The earliest visual symptom of the disease reported was in 18-month-old palms (Anon., 1981). However, the time lag between infection and manifestation of symptoms vary considerably (Nagaraj and Menon, 1956; Shanta *et al.*,1964; Mathen *et al.*; 1976). Attempts were made to develop reliable diagnostic tests which could detect the palms at very early stages of infection even before the visual symptoms are apparent.

A colour test developed based on differential dehydrogenase activity of leaf tissues was not useful in the diagnosis of the disease (Joseph and Shanta, 1963). Accumulation of free amino acids especially arginine and other ninhydrin positive free amino acids in the tender leaves of diseased coconut palms indicated the possibility of developing a colour test. However, this again gave inconsistent results under varying environmental conditions (Pillai and Shanta, 1965).

A third test based on tannin content was tried following the observation that diseased palms in general had low tannin content in the leaves, although tannin or similar colouring substances get gradually depleted as disease progresses (Lal, 1968). The change was not so marked on the onset of the disease to use it as a diagnostic tool.

A collaborative project of the Indian Space Research Organisation and the Indian Agricultural Research Institute with NASA of U.S.A. was undertaken for the early detection of root (wilt) disease by Remote Sensing Technique using false infra-red aerial photography. The findings by and large indicated that the crown of healthy palms appeared red and those of diseased palms showed paleness as a result of weaker infra-red reflectance on the film as measured by microphotometer. This method could not be used as a diagnostic tool for want of adequate data on ground level (Dakshinamurthy *et al.*, 1971; Dakshinamurthy and Summanwar, 1972).

A biochemical test to detect root (wilt) disease of coconut was developed using ethylene diamine tetra acetic acid (EDTA) as extractant of biologically active organic constituents/pigments present in the diseased palms (Dwivedi *et al.*, 1977). But, this did not give consistent results (Rajagopal *et al.*, 1988).

All these biochemical tests investigated so far were based on altered host metabolism perceptible in the form of either accumulation or depletion of substances consequent to differential enzymatic activity which could be induced under varying conditions. Shanta (1971) observed that agglutination tests were unreliable with coconut leaf and root extracts because of non-specific reaction obtained

with normal serum proteins. Similarly, non-specific reactions were obtained with diseased coconut leaf extracts against all the tested antisera in Ouchterlony's double diffusion test.

A sero-diagnostic test developed by Solomon *et al.*, (1983) and a physiological test standardised by Rajagopal *et al.*, (1986) proved to be more consistent in detecting the disease much before the visual symptoms appear.

The Agar Gel Double Diffusion Test (Solomon *et al.*, 1983) could be used to detect the disease in palms with certainty irrespective of their age group and soil type. The antiserum is specific to root (wilt) disease and it doesn't react with samples from healthy and budrot affected palms (Fig. 13).

Common antibodies from the root (wilt) antiserum were removed by intragel cross absorption technique (Regenmortel, 1967). Since the technique is time-consuming (about 96 hours), a rapid method has been standardised on the basis of serum cross absorption. Total protein isolated from the spear leaves of healthy palms was used for absorbing host antibodies from root (wilt) antiserum. The cross absorbed antiserum could be used for screening samples. By this techique 50% saving in time could be achieved (Anon., 1985a). The test is found to be in agreement with visual identification of diseased palms upto 95.3% (Table 9).

The severity of the disease also could be determined based on the intensity of reaction, the intensity being highly pronounced in the early stage of disease followed by a fall in the intensity as the disease progresses. In all tests irrespective

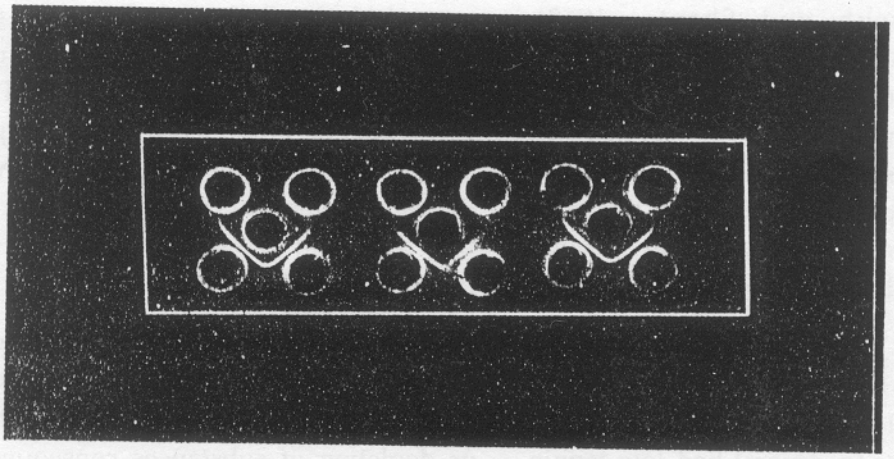

Fig. 13 Double diffusion test

Centre well : Root (wilt) antiserum

Upper well : Leaf extracts from healthy palms

Lower well : Leaf extracts from diseased palms

Table 9. Serological reaction of samples from diseased palms

Year	No. of samples tested	No. of samples with positive reaction
1976	161	158
1977	26	25
1978	30	30
1979	38	38
1980	43	41
1981	--	--
1982	51	50
1983	35	34
1984	92	79
1985	44	42
Total	520	497
Percentage		95.3

of the soil type in which palms are being grown, the antiserum reacted against diseased samples (Table 10). It has been shown based on serological test

Table 10. Precipitin reaction of root (wilt) antiserum against healthy and diseased palms from different soil types

Condition of palm	Soil type	No. of samples tested	No. of samples reacted
Healthy	Laterite	90	Nil
"	Alluvial	60	Nil
"	Sandy loam	58	Nil
"	Clayey	40	Nil
Diseased	Laterite	112	108
"	Sandy loam	921	898
"	Reclaimed Sandy loam	58	56

(Anon., 1985 a) that the time lag between the detection of latent stage of the disease and manifestation of visual symptoms varied from 6 to 24 months.

Enzyme Linked Immunosorbent Assay (ELISA), a more sensitive and rapid diagnostic test, has been standardised for the quick detection of root (wilt) disease (Anon., 1996). Plate coated indirect ELISA is performed with crude leaf extracts of coconut, root (wilt) antibody, Horse radish peroxidase as enzyme conjugate and tetra methyl benzidine as the substrate. Samples of diseased palms recorded four times higher absorbance value over healthy samples. The test could be completed within 44 h and can be used to screen atleast 36 samples at a time using microlitre quantity of antiserum. The serological test is extensively used for confirming the health status of apparently healthy high yielding palms identified in the hot spot area of Alappuzha, Kottayam, Kollam and Pathanamthitta districts. Of the 2304 samples disease status (Table 11).

The stomatal resistance and transpiration rate of last fully opened leaf next to the spear leaf were determined with Li-Cor 1600 steady state porometer (Rajagopal *et al.*, 1982; Rajagopal *et al.*, 1986). Studies on diurnal fluctuations (6 to 18 hrs) and seasonal variations (dry and wet) in the stomatal resistance and transpiration revealed that the determinations of these parameters during the mid-day in the dry season to be the best for distinguishing the diseased palms from the apparently healthy ones. There was high stomatal

Table 11. Serological reaction of samples from elite palms

Year	No. of samples tested	No. of samples with positive reaction
1986	170	69
1987	30	9
1988	56	30
1989	50	18
1990	34	15
1991	177	33
1992	174	49
1993	222	44
1994	233	117
1995	278	173
1996	525	243
1997	190	54
1998	165	101
Total	2304	955

resistance with a correspondingly low transpiration rate in the apparently healthy palms whereas diseased palms exhibited low stomatal resistance and high transpiration rate (Table 12).

Based on the characteristic changes in the leaf water potential components of different whorls of leaves between the apparently healthy and root (wilt) diseased palms (Rajagopal *et al.*, 1987), the determination of leaf water potential was also found to be useful as a diagnostic technique for early detection of the disease (Rajagopal and Amma, 1989). The leaf water potential was lower in clearly diseased palms than in apparently healthy palms. However, there were symptomless palms which had the leaf water potential similar to diseased

Table 12. Stomatal resistance and transpiration rate in the first fully opened leaves (Rajagopal *et al.*, 1986)

Season	Status of disease	Stomatal resistance $sec.cm^{-1}$	Transpiration rate $\mu g.cm^{-2}\ S^{-1}$
Dry	Apparently healthy	14.33	0.053
	Diseased	4.52	0.189
Wet	Apparently healthy	1.82	0.233
	Diseased	1.58	0.300

palms and hence were 'suspected' to be diseased. All such suspected palms developed the foliar symptoms in about 14 months.

A comparative study was undertaken between the serological and physiological tests. At the start of the experiment 19 palms had visual symptoms of the disease and reacted positively to serological test and showed low stomatal resistance and low leaf water potential (Table 13). The foliar symptoms were absent in the other 25 palms, out of which only 9 palms turned out to be free of latent infection (at that given time) based on serological negative reaction and high stomatal resistance indicative of healthy nature of palms (Rajagopal *et al.*, 1987). There were 16 palms which did not show foliar symptoms but had positive serological reaction and low stomatal resistance and high water potential. These 16 palms were designated as disease suspects.

Table 13. Serological reaction (spindle leaf) and stomatal resistance and leaf water potential (LWP) (middle leaf) from a total of 44 palms

No. of palms observed	Visual symptoms	Serological reaction	Stomatal resistance sec.cm-1 (range)	LWT Bars	Remarks
19	Present	Positive	2.3-3.9 0.13	-2.15	Diseased
9	Absent	Negative	5.8-9.1 0.17	-1.64 healthy	Apparently
16	Absent	Positive	2.6-4.4	-2.24 0.28	'Suspects'

Table 14. Appearance of visual symptoms in palms subjected earlier to diagnostic tests

	No of palms with disease symptoms in months after tests.						
No. of palms observed	2	4	6	8	10	12	14
16 Disease Suspects	Nil	Nil	Nil	Nil	3	9	14
09 Apparantly Heathy	Nil	Nil	Nil	Nil	Nil	3*	1*

* These four palms had shown low stomatal resistance and LWP between 2nd and 3rd month.

The development of disease symptoms was monitored regularly in both 'suspects' and apparently healthy palms for 18 months. Table 14 shows the time taken for the appearance of visual symptoms. While it took 10 months for three disease suspect palms to exhibit the symptoms within the next two to four months (i.e. total of 12 to 14 months) the remaining palms, nine and four respectively, also had developed the characteristic symptoms of the disease. The disease index of these palms ranged from 15% to 31% i.e. early to middle stage of the disease. The fact that four of the apparently healthy palms had also contracted the disease in about 12 to 14 months preceded by low stomatal resistance and water potential reveal that periodic diagnosis of palms would be beneficial in ascertaining the latent infection of the disease.

It is thus clear that the two parameters of water relations and sero-diagnostic test could compliment each other in detecting the diseased palms prior to manifestation of visual symptoms.

REFERENCES

ANONYMOUS, 1981. Review of research on coconut root (wilt) disease. Central Plantation Crops Research Institute, Regional Station, Kayangulam. pp. 90

ANONYMOUS, 1985a. Annual Report for 1983, Central Plantation Crops Research Institute, Kasaragod, India. pp. 249.

ANONYMOUS, 1985b. Annual Report for 1984. Central Plantation Crops Research Institute, Kasaragod, India. pp. 211.

DAKSHINAMOORTHY, C., KRISHNAMOORTHY, B., SUMMANWAR, A.S., SHANTA, P. and PISHARODY, P.R. 1971. Preliminary investigation on the root (wilt) disease in coconut palms in Kerala State (India) by remote sensing technique. Proc. 21st Astronant. Cong. North Holland, 1971 : 850-854.

DAKSHINAMOORTHY, C. and SUMMANWAR, A.S. 1972. Remote sensing of coconut palm in Kerala (India). *8th Internat. Symp. on remote sensing of environment* University of Michigan Ann. Arbor. Mich. U.S.A.

DWIVEDI, R.S., MATHEW, C., RAY, P.K., AMMA, B.S.K. and NINAN, S. 1977. Rapid biochemical test to detect root (wilt) disease of coconut. *Cur. Sci.* 46: 611-612.

JOSEPH, T. and SHANTA, P. 1963. Studies on a colour test for the root (wilt) disease of coconut. *Proc. 50th Indian Sci. Cong. Pt.* III. Abstracts 618-619.

LAL, S.B. 1968. Root (wilt) disease-Resume of work done since 1964. *Proc. 3rd Session FAO Tech. Wkg. Pty. Cocon. Prod. Prot. Process Jakarta* pp.11.

MATHEN, K., PILLAI, N.G., MATHEW, A.S. and SHANTA, P. 1976. Reproduction of symptoms of coconut root (wilt) disease in potted seedings. *J. Plantn. Crops* 4: 78-79.

NAGARAJ, A.N. and MENON, K.P.V. 1956. Note on the etiology of the root (wilt) disease of coconut palm in Travancore-Cochin. *Indian Cocon. J.* 9: 161-165.

PILLAI, N.G. and SHANTA, P. 1965. Free amino acids in coconut palms affected by root (wilt) disease. *Cur. Sci. 34 : 630-637.*

RAJAGOPAL, V. and AMMA, B.S.K. 1989. Identification of root (wilt) diseased coconut palms before visual symptom expression. *CORD* 5 (2) : 77-88.

RAJAGOPAL, V., AMMA, B.S.K. and PATIL, K.D. 1987. Water relations of coconut palms affected with root (wilt) disease. *New Phytol.* 105: 289-293.

RAJAGOPAL, V., MATHEW, C. and VARKEY, T. 1982. Determination of stomatal resistance and transpiration rate in the leaves of coconut using the steady state porometer. In : *Proc. Treephysindia* (Eds. M.R. Sethuraj *et al.*), RRII, KOTTAYAM. pp. 100-104.

RAJAGOPAL, V., PATIL, K.D. and AMMA, B.S.K. 1986. Abnormal stomatal opening in coconut palms affected with root (wilt) disease. *J. Exp. Bot.* **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. *Phil. J. Cocon. Studies* **13 (2)** : 31-35.

REGENMORTEL, VAN, M.H.V. 1967. Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. *Virology* **31** : 467-480.

SHANTA, P. 1971. Studies on transmission and nature of the pathogen associated with root (wilt) disease of coconut of Kerala. *Internatl. Symp. on Pathological Wilting in Plants*, Madras.

SHANTA, P., JOSEPH, T. and LAL, S.B. 1964. Transmission of root (wilt) disease of coconut. *Indian Cocon. J.* **18** : 25-28.

SOLOMON, J.J., SASIKALA, M. and SHANTA, P. 1983. A serological test for the detection of root (wilt) disease of coconut. In : *Coconut Research and Development* (Ed. N.M. Nayar) Wiley Eastern Ltd., New Delhi. pp. 401-405.