

HISTOPATHOLOGICAL STUDIES

M.P. GOVINDANKUTTY
Central Plantation Crops Research Institute
Regional Station, Kayangulam
Krishnapuram-690 533, Kerala

Histopathological studies provide information on the initial stages of disease development, such as the process of infection, the types of cells and tissues primarily affected as well as the course of tissue degeneration and host responses. Histological and histochemical changes occur in coconut also with the development of root (wilt) disease also. In palms water and nutrients are transported through the primary vascular tissues which once developed should remain functional throughout the life of the massive plants. Information available on the general anatomy of healthy coconut tissues has been presented by Patel (1958), Menon and Pandalai (1958), and Tomlinson (1961). Only very few histological comparisons have been carried out on disease affected palm tissues.

ANATOMY OF PINNAE

Degeneration of the chloroplastids in the mesophyll cells of root (wilt) affected palms was reported by Shanta, Thommen and Menon (1959) although such degeneration, according to these authors, was observed in palms affected by physiological yellowing as well. Thomas Joseph and Shanta (1964) investigated the anatomy of tender unopened leaves of healthy and root (wilt) affected palms. According to them the stomata on the abaxial side of leaflets are slightly narrower in the diseased than in the healthy which corresponded to the general stunting of the epidermal cells. This resulted in higher percentage distribution of stomata per unit area, in the leaflets of diseased palms. The thickness of cuticle on the adaxial surface was considerably reduced in the diseased. Changes were also observed in the number of epidermal cells per unit area which resulted in the apparent stunting of the leaflets. Transverse divisions of upper epidermal cells in the diseased were accelerated and longitudinal divisions were curtailed. It was concluded that changes in the rate of divisions in the longitudinal and transverse plains subsequently resulted in slight downward curling of leaflets.

The wall thickness of cells in mechanical sclerenchymatous fibres and bundle sheaths gets reduced, which brings down the rigidity in the leaflets in hypodermis of disease affected trees. The average area occupied by xylem in the disease affected leaflets was $130 \mu \times 110 \mu$ as against an area of $1666 \mu \times 119 \mu$ in the healthy ones. Xylem vessels were also found to be slightly smaller in the diseased. Considerable changes were seen in phloem of the main bundles of disease affected palms. In an average area of $324 \mu \times 160 \mu$ occupied by phloem per field of observation, the number of cells were 191 in the healthy. The corresponding number in the diseased was 230 confined to $300 \mu \times 160 \mu$.

Mature leaflets were thinner (measuring 265-296 μ in cross section) in palms of diseased-advanced stage as against the disease-free palms (365-380 μ). These leaflets showed thinner mesophyll layers and narrow vascular bundles. Leaflets exhibited poor deposition of lignin and suberin in diseased materials. Certain leaflet bundles showed phloem necrosis. Root (wilt) disease is often superimposed by leaf rot disease (Drechslera halodes). Sections of leaflets from such palms revealed fungal hyphae in the vascular and ground tissues (Govindankutty, 1979; Govindankutty and Radha, 1977).

ROOT ANATOMY

Internal browning of the root vascular elements extending upto the cortex, and at times accompanied by disintegration of vascular tissues in about 60 per cent of the roots of the diseased palms was reported by Indira and Kamadasan (1968). Mild internal browning was recorded in 33 per cent of roots of apparently healthy palms growing in diseased soil. Development of tyloses was also noticed in the vessels of majority of roots. None of the above derangements, according to these workers was observed in the tissues of healthy palms. Subsequent studies, however, failed to demonstrate vascular browning in externally healthy roots from disease affected palms in the same locality (Dwivedi et al., 1978).

Recently further observations on the anatomy of roots were reported (Govindankutty and Vellaichamy, 1976; Govindankutty and Radha, 1977). In these studies, externally healthy roots from trees in the apparently healthy and different stages of root (wilt) disease, categorised based on the diagnostic

.../-

symptoms described by Radha and Lal (1972) and the disease index proposed by George and Radha (1973) were employed. Root damage was obviously high in palms of disease prevalent area, and there occurred a progressive deterioration in the root system through increased die back of roots and non-specific root rot. Other investigations designed to determine the per cent of root rot had revealed 9-64 per cent in apparently healthy and 12-90 per cent in diseased seedlings (Radha, Potti and George, 1973). In the recent studies there were indications of retardation of meristematic activity, and certain stunted roots from palms in early stages of disease showed abnormally hypertrophied cells in place of metaxylem elements, which suggested that dedifferentiation or anomalies in vascular differentiation was concomittant with stunting of root tips. Certain roots from apparently healthy and evidently diseased trees exhibited poor staining of mechanical and vascular tissues.

Walls of the metaxylem elements appeared disorganised and thin. Reduction in wall thickness was contributory to the uneven shape of walls. In transections the biggest xylem element in some diseased samples measured 370-440 μ long and 270-285 μ wide, whereas some healthy samples showed dimensions of 480 μ x 315 μ . Tyloses were encountered in 16/126 roots of 21 apparently healthy, 15/72 roots from 12 diseased early and 13/48 roots of 8 diseased advanced palms examined. Tyloses developed by the invagination of the protoplast of the surrounding xylem parenchyma into the lumen of the xylem vessels through pits. Some of these developed lignification like xylary elements and exhibited reticulate pattern. Gummosis was noticed in xylem vessels in several roots which may be regarded as an evidence for upset in the carbohydrate metabolism.

Metaxylem vessels of some roots showed fungal hyphae generally around the interxylary septa. In some cases the bordering parenchyma cells were also found to harbour these. Such hyphae were encountered in 20/126 roots of 21 apparently healthy, 13/72 roots of 12 diseased early and 20/48 roots of 8 severely affected palms. Fungi like Rhizoctonia bataticola, R. sclani, Botryodiplodia theobromae, Fusarium equiseti, Cylindrocarpum effusum etc. are reported to have been isolated from coconut palms in root (wilt) disease prevalent gardens (Anonymous, 1976).

.../-

In the roots of coconut palms of all ages and categories of root (wilt) disease in the disease prevalent area phloem tissues showed increased chromophily and necrotic obliteration. Degenerative changes were chiefly confined to the protophloem and early metaphloem. Complete blocking of the sieve element members was not detected in any specimen. The cell walls of phloem of such materials were disintegrated in a few instances. Phloem degeneration occurred in 24/126 roots of apparently healthy, 26/72 roots of diseased early and 14/48 roots of diseased-advanced. Also, ultrathin section of plastic embedded coconut tissues prepared for electron microscopy revealed signs of accumulation of gum. Gummosis was more prominent in xylery tissues.

Burrowing nematode, Radopholus similis infestation is very common in the root (wilt) recorded and neighbouring areas. Root infection by this nematode can be made out by the longitudinal brown lesions on the root exodermis. Incipient lesions of this nematode appear as water soaked streaks and the borders of these later developed brown colour. Microscopic preparations of root tissues with lesions showed the nematodes 15-20 cell layers deep in the cortex. Nematodes were seen in inter and intracellular positions in cortex and they were never found to colonize near the stelar cells. Burrows or galleries produced in the cortical tissues were found to contain nematodes and their eggs. Cells around the site of lesions retained intense stain indicating alterations in chemical activities. Suberised and thick walled tissues later developed around these lesions. Incipient lesions were seen in 44/126 roots of the 21 apparently healthy, 27/72 roots of 12 diseased early and 16/48 roots of 8 diseased advanced palms studied (Govindankutty and Vellaichamy, 1976).

HISTOCHEMICAL STUDIES

Histochemical localization of metabolites was attempted on root tissues from healthy and diseased (advanced) categories (Govindankutty, 1979). Staining reactions for total carbohydrates (iodine-potassium iodide reaction) and insoluble polysaccharides (periodic acid - Schiff and alcian blue tests) revealed reduction in concentrations of starch in root cortical cells and polysaccharide materials on cell walls in the samples

.../-

from the diseased category. Polyphenolic tannins (localized by ferric chloride test) also tended to be in lower concentration in the diseased palms. Burrowing nematode lesions, however, showed accumulation of phenolic substances. Nematode burrows had deeply staining cells and suberised tissues delimited the cortical lesions. Staining reactions for DNA and RNA (methyl green-pyronin method; azure B and Feulgen reactions) did not show any appreciable differences between the healthy and pathological tissues. Attempts to demonstrate proteins (naphthol blue black and bromophenol blue methods) showed reduced intensities in the diseased roots. Lignification (stained with phloroglucinol-HCl) was reduced in the diseased roots but in some cases the inner cortical cells were deeply staining. Suberisation was also affected in the diseased palms.

Sections of pinnae showed reduction in suberisation and lignification. Carbohydrate localization attempts on pinnae showed strong reaction in the leaf mesophyll of diseased palms.

Histological alterations and anomalies were met with in apparently healthy palms from root (wilt) disease prevalent gardens which suggest that these palms had contracted some infection long before foliar (diagnostic) symptoms manifest. No information is available on the histopathology of seedlings artificially inoculated with the different microorganisms and nematodes isolated from the tissues of root (wilt) affected coconut. ^{A pathogenicity experiment} is being initiated, which would provide information as to which of these alterations are associated with a particular organism. Histochemical studies should also be pursued which would make the host-pathogen interactions more meaningful by revealing the trends of initial chemical changes within the susceptible palm.

REFERENCES

- ANONYMOUS, 1976. Coconut diseases of uncertain etiology. Tech. Bull. 1, Central Plantation Crops Research Institute, Kasaragod, 30 pp.
- DWIVEDI, R. SNEHI, POTTI, V.P., SUNATHYKUTTY AMMA, B., GOVINDANEUTTY, M.P., SOLOMON, J.J. and JAYASANKAR, N.P. 1978. Investigations on vascular browning in the roots of root (wilt) diseased coconut (Cocos nucifera Linn.) Curr. Sci., 47: 31-32

- GEORGE, M.V. and RADHA, K. 1973. Computation of disease index of root (wilt) disease of coconut. Indian J. agric. Sci. 43: 366-370
- GOVINDANKUTTY, M.P. 1979. Histopathological studies in relation to root (wilt) disease. pp. 53-54 In: Central Plantation Crops Research Institute - Annual Report for 1977, Kasaragod
- GOVINDANKUTTY, M.P. and RADHA, K. 1977. Symptomatology of root (wilt) disease pp. 56-57 In: Central Plantation Crops Research Institute - Annual Report for 1976, Kasaragod
- GOVINDANKUTTY, M.P. and VELLAICHAMY, K. 1976. Histopathology of the roots of coconut palms affected with root (wilt) disease. Paper presented at Int. Symp. on Coconut Res. Dev., Dec. 1976, Kasaragod (p. 46 In: Abstracts of papers)
- GOVINDANKUTTY, M.P., VELLAICHAMY, K., RADHA, K. and POTTI, V.P. 1976. Symptomatology of root (wilt) disease p. 47 In: Central Plantation Crops Research Institute - Annual Report for 1975, Kasaragod
- INDIRA, P. and RAMADASAN, A. 1968. A note on the anatomical derangement in the root (wilt) diseased coconut palm. Curr. Sci. 37: 290-292
- MENON, K.P.V. and PANDALAI, K.M. 1958. The Coconut Palm - A Monograph. Indian Central Coconut Committee, Ernakulam, 384 pp.
- PATEL, J.S. 1938. The Coconut - A Monograph, Government Press, Madras, 313 pp.
- RADHA, K. and LAL, S.B. 1972. Diagnostic symptoms of root (wilt) disease of coconut. Indian J. agric. Sci. 42: 410-413
- RADHA, K. POTTI, V.P. and GEORGE, M.V. 1973. Symptomatology of root (wilt) disease. p. 56 In: Central Plantation Crops Research Institute - Annual Report for 1972, Kasaragod
- SIHANTA, P., THOMMEN, K.J. and MENON, K.P.V. 1959. Studies on the anatomical changes in the tissues of coconut palms affected by root (wilt) disease. I. A note on the changes in the mesophyll. pp. 272-276 In: Proc. 1st Conf. Coconut Res. Workers, Trivandrum
- THOMAS JOSEPH and SIHANTA, P. 1963. Anatomical changes in the tissues of tender leaves of coconut palm affected by the root (wilt) disease. J. Indian bot. Soc. 42: 61-65
- TOMLINSON, P.B. 1961. Anatomy of the Monocotyledons - II. Palmae. Oxford University Press, London, 453 pp.