Histopathology of Roots of Coconut Palm Affected with Root (wilt) Disease

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Abstract

Anatomy of roots from healthy and diseased coconut palms was studied using serial microtome and free-hand sections. Some externally healthy roots from apparently healthy, and obviously diseased trees (naturally infected) from an area of active disease spread showed poorly staining mechanical and vascular tissues. Walls of tracheal elements were thinner and disorganized. Tyloses occurred occasionally in metaxylem vessels. Several roots revealed degenerate phloem. Necrotic effects were shown by abnormally and deeply staining phloem with dense contents. Tissue degeneration was less pronounced in metaphloem. Many healthy-looking roots from apparently healthy and diseased palms had fungal hyphae (?) in metaxylem. Sections of roots with external lesions of the burrowing nematode *Radopholus similis* revealed the presence of nematodes and their eggs in cortical burrows. Such burrows contained deeply staining cells encircled by abnormal sclerenchyma. Degenerative changes in conducting elements could derange the flow of nutrients and thereby contribute to root death due to starvation.

Introduction

The root (wilt) diseased coconut palms exhibit macroscopic symptoms characterized by general drooping, yellowing, and necrosis of leaflets. The number of roots is reduced and a high percentage of the roots show rotting (Anonymous, 1976). External symptoms of diseases are the manifestations of biochemical and histological alterations occurring in the susceptible host caused by or as an effect of pathogenic infection. Association of several microorganisms with the root (wilt) disease affected palms has been reported (Anonymous, 1976).

Anatomical studies on root (wilt) affected coconut palms have been attempted by Joseph and Shanta (1963) and Indira and Ramadasan (1968). The present studies were undertaken to gather additional information on the histological alterations in the roots of coconut.

Materials and Methods

Roots of the coconut cultivar West Coast Tall were collected from the bole regions of healthy palms from a disease-free area (Kasaragod) and apparently healthy and diseased palms from a severely infected area (Kayangulam). Samples with lesions at the burrowing nematode were also collected at Kayangulam.

Roots without obvious external damage were fixed in FAA (formalin-acetic acid-ethanol). For microtomy, materials were dehydrated and infiltrated in a tertiary butanol series. Serial transections (10-15 μ thick) and free-hand preparations were used. Johansen's safranine fast green, resorcinol blue and acid fuchsin-lactophenol were used for staining. Some sections were stained with phloroglucinol-HCI to test for lignification, and Sudan IV to determine suberization (cf., Johansen, 1940). Observations were recorded from at least six roots from each palm.

Results

The structure of the root fits the description of Tomlinson (1961). However, for the sake of comparing the histological alterations in the disease affected palm, the following details are given. The root has no epidermis in a mature differentiated zone. A conspicuous exodermis develops in place of the worn out epidermal layer. The cortex has schizogenous cavities. The inner cortex gets sclerotized as the root matures. An endodermal cell with casparian bands delimits the cortex. This encloses a pericycle having passage cells and cells with reticulate thickenings. Tanniniferous cells are interspersed in cortex, xylem parenchyma, and pith cells.

The xylem and phloem occur in different radial bundles. Some roots have 40-44 protoxylem points and an equal number of phloem. Metaxylem vessels form an inner ring. The metaxylem in a representative sample measures 480×315 μ. Phloem bundles here occupy an area of 315×120 μ. The large metaphloem measures 90×48 μ. The pith in old root is lacunose.

Root damage is quite obvious in disease prevalent areas. There occurs a progressive deterioration in the root system through increased die-back of roots and root rot. Roots are very few in palms at the advanced stages of the disease. This may be because of a reduction in meristematic activity. Certain roots from apparently healthy and obviously diseased palms showed poorly staining mechanical and xylem tissues. The reduced intensity of staining is indicative of poor lignification. However, in some cases, the inner cortical cells were deeply staining.

Walls of metaxylem elements become disorganised and thin. Their wall thickness was much reduced and resulted in uneven walls. The biggest metaxylem in some diseased roots measured 370-440 μ long and 270-285 μ wide. Tyloses were seen in 18/132 roots examined. They are formed by the invagination of the protoplast from the bordering parenchyma into xylem lumen through pits in the walls of xylem. Some tyloses had undergone reticulate thickenings.

Metaxylem of some roots contained fungal hyphae-like structures. They were noticed in 10% roots of apparently healthy palms, 17% of diseased early palms, and 25% roots of diseased advanced palms. Such structures occurred generally around the septa of adjacent xylem members. In a few cases the bordering parenchyma cells were also found to contain these indicating colonization of xylem vessels by some fungi. We did not attempt to identify them.

Phloem tissues showed degeneration in the roots of palms of all categories from the disease prevalent area. The cells of protophloem and a few members of metaphloem showed necrotic effects. The component cells were smaller in size. They had an unusually intense chromophilic reaction and dense contents. The phloem walls in such specimens were distorted and collapsed. Complete blocking of the phloem was not seen in any specimen. Callose accumulations were found in some sieve plates. Degeneration of phloem was noticed in the roots of apparently healthy (13%), diseased early (19%), and diseased advanced (29%) palms. Some roots had degenerate phloem and abnormal cells in place of metaxylem vessels.

Burrowing nematode lesions are often encountered in the roots. Incipient lesions appear as water soaked areas. The borders of lesions attain a brownish colour and longitudinal cracks appear in the exodermis. Nemas were seen 15-20 cell layers deep in cortex. They however, were not seen to penetrate the steel. Nematodes were seen embedded between and inside cells. Galleries produced by destruction of cell walls were found to contain nematodes and their eggs. Invasion occurs also along the points of lateral root emergence. The cells at the site of lesions take up more fast green stain than the bordering tissues. Suberized tissues develop around the lesions. Nematodes in incipient lesions were encountered in 32% roots of apparently healthy, 27% roots of diseased-early, and 33% roots of diseased-advanced palms.

Discussion

Several microscopic changes were seen in the roots of palms growing in disease affected gardens. The histological alterations seen in the undoubtedly diseased palms were present also in some roots of apparently healthy palms. This suggests that they might have contracted some infection long before visual symptoms are manifest. Tissue breakdown should be a major factor in the weakening of the root system. *Radopholus similis* is known to produce extensive damage to crops like citrus (Du Charme, 1959). The fungi which colonize the vessels were not identified.

Vessel blockage in plants has been attributed to fungus hyphae, spores, bacterial cells, organic substances etc. (Beckman, 1964). Indira and Ramadasan (1968) had reported the presence of tyloses in majority of the roots in an earlier study. They could be produced in vessel members as a result of some infection or as a defence mechanism.

The observation of phloem degeneration was noteworthy. Phloem necrosis has long been recognized as a symptom of virus diseases (Esau, 1938). Incidentally, the mycoplasma-like organisms associated with lethal yellowing and Kaincope diseases of coconut are located in the phloem tissues (Parthasarathy, 1973; Dollet and Giannotti, 1976). Dutch elm disease, possibly caused by a mycoplasma also produces phloem necrosis (Braun and Sinclair, 1976). Phloem necrosis has been observed in the apical meristem of cadang-cadang affected coconut palms (Rasa, 1968). We are tempted to believe that in the case of coconut root (wilt) disease, the degenerative changes observed in the conducting elements would derange the flow of nutrients and thereby contribute to root death following starvation.

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Discussions

Asana : In a paper read yesterday, a case was made out for micronutrients being involved in this disease. Micronutrient deficiencies are known to cause several morphological and histological deformities. To what extent, do you think, would micronutrient deficiencies be responsible for the changes described by you ?

Govindankutty : Histological alterations are induced by the application of chemicals like alar, kinetin, and auxins. The role of micronutrients in producing the histological symptoms described here is worth testing.

Nirula : Did you come across necrosis caused by burrowing nematode in roots collected from Kasaragod area ? What was its incidence as compared to those on roots collected from diseased areas ?

Govindankutty : A low population of *Radopholus* is reported to occur in Kasaragod also. *Radopholus* is capable of producing similar histological changes in certain other crops too. The present studies were confined to burrowing nematode infected coconut materials from Kayangulam.