

WORKING PAPER NO. XXVIII

ADVANCES IN RESEARCH ON THE ROOT (WILT) DISEASE
PROBLEM OF KERALA, INDIA

by

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INTRODUCTION

The important fact that was reported at the last meeting of this Working Party, and which is a very characteristic feature of the root (wilt) disease of Kerala, is its slow spread. This is the most encouraging aspect of the disease. Although the disease has crossed both the northern as well as the southern outposts in Kerala, the spread has still been slow and the total area affected is not much larger now than what was reported in 1961.

Symptoms

The main symptoms of the disease are flaccidity or ribbing of leaflets accompanied by an abnormal bending of petiole, which often breaks, and slow wilting of trees. The outer whorls of leaves often turn yellow and the buttons fall abnormally. The leaflets show tip and marginal necrosis. A constant decline in yield sets in till the tree finally becomes barren. The wilting is associated with root rot and is a characteristic feature due to which the disease carries its name.

The susceptibility of coconut palm to the disease is influenced by age (Table 1). Seedlings below 4 years of age are not affected while the most vulnerable stage of the palms to the disease is at the bearing age (6-15 years). Progress of disease in young palms (6-15 years) is rapid, leading to deterioration within 3-4 years after initial symptoms, while it is slow in middle-aged or older palms (35-50 years), taking 8-10 years for deterioration to set in. The yield of young affected palms is also severely affected as compared to that of the middle aged ones, which do not suffer to any appreciable extent for a long time.

The losses due to the disease are on the increase particularly because (1) the disease has spread to wider areas and to newer plantations in infected gardens and (2) the younger plantations (underplantings and replantings) on infection are showing faster deterioration.

Nutritional and biochemical investigations

Soil survey: As reported earlier the disease has been found to occur on all the soil types of Kerala. The disease is more severe in poorly aerated soils and conditions of waterlogging and poor drainage probably predispose the palm to infection.

The nutrient status of the soils from healthy and diseased area essentially had no differences except that the diseased soils were poorer in calcium, available potash, total exchangeable bases and low in pH. The occurrence of disease in rich clayey reclaimed soils however points out that soil aeration is an equally important factor. This is supported by the observation that the disease is more common in extensive reclaimed gardens than on the bunds where the ebb and flow of the water aerates the root zone.

Pot culture experiments have shown that soluble iron, manganese, ammoniacal nitrogen, and oxidisable matter were found to accumulate under waterlogged conditions in the soil. Leaf analysis indicated that the uptake of major nutrients was inhibited.

Tissue analysis

A detailed study on the metabolism of proteins and enzymes, amino acids, organic acids, carbohydrates, lipids, major and minor nutrients in the ontogenic stages of different parts of the palm under healthy and diseased conditions as also under different environmental conditions and manurial treatments is in progress. Preliminary studies indicated increased catalase activity and accumulation of amino acids and non-volatile organic acids in tender leaves of diseased palms. Chromatographic studies showed varietal variations in amino acid contents of pollen and leaf tissues.

Nutritional studies

The results of the NPK manurial experiments conducted at the Central Coconut Research Station, Karyangulam, to ascertain their effect on the incidence and development of disease, have shown that the NPK treatments in higher dosages did not improve the foliar condition or the yield of the adult diseased palms. The application of NPK also did not prevent the incidence of disease in young palms at pre-bearing age. The major nutrients when applied as foliar sprays did not prove better than soil application of nutrients. The palms both under soil and foliar spray applications did not resist the incidence or progress of disease.

A pot culture experiment showed that deficiency symptoms of major elements on seedlings failed to reveal any similarity with the disease syndrome. Results also showed that nitrogen plays a more

important role than that of phosphorus or potassium. Phosphorus probably enhances the uptake of potassium.

Studies on the effect of certain activated fertilisers like plantomine, fenugol and trigolene did not yield any encouraging results with infected palms. Trials with proprietary products like steracal green label and sea magic are being continued.

Application of sulphur in the form of elemental sulphur, calcium sulphate and magnesium sulphate over a basal dressing of N, P, K manures was found to bring increased yield and improvement in foliar conditions in diseased palms (Table 2).

A micronutrient experiment on 384 palms in the field was conducted at the Central Coconut Research Station, Kayangulan, for 10 years. The nutrients were applied for the first 5 years as soil application and for the rest of the period as root injection. The experiment has indicated that the micronutrients singly or in combination applied to diseased palms had no curative effect. Since the beginning of the experiment in 1953, 50% of the palms under the treatments got infected. A general increase in the yield of Mg treated palms, irrespective of whether they were healthy or diseased, was obtained.

Pathogenic studies

Mycological work: As reported earlier, Rhizoctonia bataticola and R. solani were found associated with root-rot which is a characteristic symptom of the root (wilt) disease.

In view of the earlier suggestion that fungal infection is secondary, studies on the predisposing factors conducive to the infectivity of R. solani and R. bataticola were carried out. Primary inoculation of young palms in the field with the virus increased infectivity of Rhizoctonia spp. in the roots (Table 3). Among the uninoculated palms, those showing symptoms of disease proved to be more susceptible to fungal infection. Preliminary studies on the correlation between fungal infection and carbohydrate content of roots indicated that the roots of healthy palms were richer in carbohydrate than those of diseased ones. (Table 4).

Evaluation of the soil and rhizosphere microflora of coconut palm from healthy and diseased areas failed to reveal the association of any organism other than Rhizoctonia spp. with the occurrence of disease. The quantitative reduction in the rhizosphere microflora in the diseased palms appeared to be inconsistent with variation in soil type and depth of sampling. In sandy loam soil reduction in microbiological activity in the soil in the root zone of diseased palms was noticed.

Studies on the root system of palms in different stages of disease were carried out. Although an increased production of fresh roots was sometimes induced in affected trees, it decreased with advance in disease. No correlation between root rot and initiation of primary symptoms of disease was observed whereas the degree of root rot and the severity of disease had a positive correlation. The seedlings in sterilised soil in the insect proof house did not show any root rot in spite of the foliar symptoms of flaccidity, yellowing and stunting of leaves. These seedlings have also not developed necrosis of foliage till the fourth year of their infection. This points out a possible association of the root rot with soil fungi in nature and also that perhaps the necrosis of leaflets is correlated with root rot or poor nutrient status of soils.

Virological studies

It was first indicated in 1956 that a virus might be involved in this disease complex. Transmission trials in the field confirmed the earlier results. The symptoms of the disease were consistently reproduced on artificial inoculation in palms of all age groups both by the abrasion method and by feeding infective adults of Stephanitis typicus Dist.

Transmission trials under insect proof conditions on 20 potted 2 year old coconut seedlings by different methods were started in 1959. Observations on the different groups of inoculated seedlings and on the two control seedlings showed that by May 1963, five of the six seedlings inoculated by the abrasion method and two of the six seedlings fed by infective S. typicus were diseased. Paling, flaccidity and slight stunting of the leaves were the symptoms exhibited by these. One of the two seedlings inoculated by the basal application of washed roots of naturally infected coconut trees also showed slight flaccidity of leaflets (Table 5). Diagnostic tests on cowpea with the 20 experimental seedlings were carried out at frequent intervals. Positive symptoms were produced with the five seedlings infected by the abrasion method, one seedling infected by S. typicus and one seedling infected by the incorporation of root bits into soil.

When seeds of cowpea were sown at the base of these test seedlings in February, 1964, 2½ years after foliar symptoms were apparent, disease was noticed in four of the sap inoculated series and two of the seedlings infected by the incorporation of root bits (Table 6).

The virus is found to be soil transmitted and soils from the base of infected coconut palms gave positive infections on cowpea while those from the healthy ones did not do so. As reported earlier, the virus occurs in the clay and fine silt fractions of infective soil.

Preliminary studies to find out the infectivity of soils at various depths and distances from the base of the diseased trees indicated that in a sandy loam soil, samples collected at 4 metres from the base of a diseased tree were infective to a depth of about 2 metres, whereas soils at about one metre distance were found to be infective at a depth of three metres (Table 7). Most roots in the root system of coconut palms occur on the surface, about 50 per cent of them being found at 2-4 metres below ground level. It would seem probable from this that soil gets contaminated by the release of virus through roots either through root exudates or by the decay of the old dead root tissue. It was further observed that unsieved soils containing dead roots etc. from the base of the diseased trees were more infective than sieved soils from which all visible particles of debris were removed.

It was observed some time earlier that the infective soil mixed in equal amounts with sterile soil loses infectivity. More tests were performed with the following proportions of the infective and sterilised soil, 4:0, 3:1, 2:2, 1:3 and 0:4 to make 2 kg. of soil per pot. Infection was noticed in cowpea seedlings grown *in situ* in 4:0, 3:1, and 2:2 soils only. Soil mixtures tested for infectivity by inoculation on cowpea, however, gave infection up to 1:3 level.

The effect of dry and wet storage of sandy loam soil collected from a diseased area at 28° to 30°C. on its infectivity was also studied. Samples of air-dried infective soil were passed through a 2 mm. sieve and stored in petri-dishes in 10 g. lots, one lot being saturated with sterile distilled water. At intervals clay and silt fractions from these samples were tested for infectivity on cowpea. The results presented in Table 8 show that the majority of samples remained infective up to 9 to 12 days.

Cowpea (*Vigna sinensis* L.) had been reported to be an indicator host for the virus. The age of seedlings which will give the best reaction and the period of symptom expression was determined by an experiment. Six to ten days old seedlings gave good results. The symptoms of necrosis of developing leaves could be seen on the 3rd or 4th day and the leaf deformities were clear by the 7th day. One to two week old cowpea seedlings raised in sterilised soil and transplanted in infective soil developed symptoms of disease 8-10 days after transplantation. No symptoms were produced on older seedlings. (Table 9). Seeds of cowpea sown in infective soil took nearly two weeks to exhibit the symptoms.

Although cowpea is a good indicator host for the virus because the reactions can be read in a short time it is found not to give consistent reaction particularly during the hot season.

A colour test based on the dehydrogenase activity of leaf tissues was developed after testing 1100 samples from 323 trees in different stages of disease. The test was standardised as follows: Samples of tender leaves are collected in the morning, 0.075 g. of each weighed out into separate vials, frozen for 4 hours, mixed with 2 ml. of 0.001 M aqueous dye, evacuated for one hour in a vacuum desiccator and incubated at 37°C. overnight. The samples were exposed to a 100 watt. light source at 30° to 32°C. the next day and the rate of decolorisation was recorded every 2 hours till 4 P.M. The results given in Table 10 indicate that the percentage of palms decolorising the dye to 50 per cent or above increased with the development of the disease and that none of the samples from the diseased trees retained the original colour. Cross inoculation tests on cowpea with leaf samples of 183 of these test palms which exhibited foliar symptoms of the disease show nearly 91% correlation with the colour test. (Table 11).

When this test was extended to different healthy as well as diseased areas, however, the results obtained were not consistent. Since this test is dependent on the enzymatic activity of the tissues which are influenced by environmental factors, it was found that the test will have to be standardised with different types of soil and for trees in different stages of development.

Infective preparations were obtained from diseased leaf sap by precipitation of proteins in 50 per cent alcoholic solution. The precipitate, suspended in distilled water or neutral phosphate buffer 1/10th the original volume of sap, was dialysed overnight in running tap water and centrifuged. The supernatant which was found to be infective was used as the antigen for injecting rabbits. Serological trials using different preparations of infective material from both coconut and cowpea were tried but no conclusive results have been obtained so far.

Analysis of the distribution of free amino acids in the tender leaves of both healthy and diseased coconut palms by two dimensional paper chromatography indicated the accumulation of aspartic acid, glycine, alanine, valine - methionine, leucine - isoleucine, proline, asparagine, lysine and arginine in the diseased tissues. The increase of the amide asparagine is comparable to the same phenomenon met with in maize rough dwarf virus, papaya mosaic, leaf roll viruses etc.

The external symptoms in tender leaves of coconut palms affected by the root (wilt) disease are correlated with various anatomical changes in the epidermal, mechanical and conducting tissues. In spite of the apparent stunting of leaves, an evident change in the rate of division of the upper epidermal cells in the longitudinal and transverse planes takes place resulting in slight downward curling of these. The comparative thinness of the cuticle on the upper epidermal surface and the increased percentage distribution of stomata per unit area contribute towards increased water loss. Failure of development of all types of sclerenchymatous tissues and phloem proliferation are the other important changes that occur

in leaves of affected palms. A slightly increased deposit of callose in the sieve plates in tender leaves of diseased palms was noticed. Observations on the cowpea leaves showed initiation of degeneration and necrosis of the phloem and the adjacent parenchymatous tissues in the diseased material, a phenomenon comparable to that observed in the diseased coconut leaves.

A number of experiments were carried out with adults of Stephanitis typicus Dist. collected from the field as well as with those bred in the laboratory to study the relationship of the virus with this insect vector.

It was observed that a minimum of one insect per cowpea was enough to produce successful infection. However, only 5 per cent of the plants with one insect feeding on each were infected. Adults of S. typicus bred in the laboratory on healthy leaves of coconut were used to study the minimum acquisition feeding period required for an adult insect to become infective. Infection was obtained from those that were fed for 2 hr. but not from those fed for 1 hr. 45 minutes. When a minimum of 10 adults per plant were used after an acquisition feeding for 24 hr., infection was obtained after a 16 hr. infection feeding. The insects were found to retain infectivity up to 22 hr. after acquiring the virus.

Even 4 to 5 year old seedlings in the early stage of disease were found to be effective sources of the virus. Trees in all age groups and in different stages of disease were infective, although trees in the middle stage of disease were more infectious than those in the early and advanced stage.

Sixteen per cent infection was obtained from a mixed sampling of insects collected from both healthy and diseased coconut seedlings, without any prior acquisition feeding. The percentage infection obtained from samples of insects collected from diseased seedlings was 45 and that from insects collected from healthy seedling was 11 per cent.

These findings indicate that S. typicus is a vector of the virus capable of spreading it in the field. The spread is accentuated by favourable factors like (1) active source of virus since diseased trees of all age groups and trees in the early stage of disease are infective (2) occurrence of the vector in large numbers on coconut trees and (3) a short acquisition feeding period of 2 hr. The spread is limited by factors such as (1) sluggish movement of the insect (2) semi-persistent nature of the virus (3) the low percentage of infective insects and (4) long incubation period of virus in the tree before symptoms become apparent and the tree becomes infective.

Physiological studies

The rate of loss of water from the surface of healthy and diseased palms was studied. It was found that the fresh weight/dry weight ratio and

the loss of weight of leaf tissue per sq. cm. per minute are significantly higher in the diseased palms than that in the corresponding healthy palms.

The organic and inorganic contents in the root-sap collected from the cut ends of roots were higher in the diseased than in the healthy palms. These studies indicate that uptake of water by a diseased palm may not have been affected much although the loss of permeability of root cells due to root damage may have led to indiscriminate entry of minerals and possibly other substances also into the shoot.

It was observed that tannin content of middle and outer leaves of diseased palms in middle and advanced stages of disease was significantly higher than that in healthy. In tender leaves the increase was not statistically significant. Failure to get consistent results in infectivity tests with diseased coconut leaf extracts on cowpea may be due to the denaturing of virus by tannins during extraction.

Disease resistance

Investigations on the heritability of the disease and disease resistant capacity of indigenous and exotic varieties from healthy areas indicate that the progenies of healthy parents in the diseased tract as well as hybrids from a healthy tract behave almost similarly when planted in a diseased tract. The percentage disease incidence was lower in these two groups as compared to the progenies of diseased parents. Among indigenous and exotic varieties tested, Chowghat dwarf, Andaman, Laccadive, Philippine, New Guinea, Cochin-China and Ceylon were found to be susceptible.

Studies on the role of polyphenols in initiating metabolic defence reactions has been started.

Studies on pattern of spread

A new project to study the pattern of spread of the disease has recently been started. Five plots representing various soil types of Kerala have been selected in areas of new spread of the disease. These plots have a single locus of infection either in a single infected tree or in a few infected palms close to a diseased one. Studies consist of testing of soil, roots and leaves of the infected and neighbouring palms and infectivity of *Stephanitis typicus* on cowpea. Analysis of soil and leaf tissue for deficient or accumulated nutrition and soil microfloral population and microbiological activity. The information is hoped to be utilised for evolving practical methods of control of spread of disease in new areas.

Control

Studies have been initiated with inhibitors of viral activity both in the tissue as well as in the soil.

Roots of cowpea seedlings raised in infective soil were surface sterilised with 0.1 per cent mercuric chloride before transplantation to sterilised soil. No infection was obtained when 3 to 5 day old seedlings were treated whereas control seedlings washed with water alone were infective. This shows that mercuric chloride possibly interferes with the infective principle at the prepenetration stage.

Experiments have been started to study the effect of chemicals, soil fungicides, nematocides etc. on infectivity of soils.

There is no direct evidence yet available to prove that the coconut root (wilt) disease is caused by a virus. Available information, however, points out that the causal organism is a biological agent similar to a virus. The slow spreading nature of the disease is encouraging and provides plenty of hope for an effective control.