13.2 Studies on the transmission and nature of the pathogen associated with the root (wilt) disease of coconut in Kerala

(Mrs.) P. Shanta,
Central Plantation Crops Research Institute,
Regional Station, Kayamkulam, Kerala, India.

The root (wilt) disease of coconut in Kerala is one of the three major diseases of this crop with an unknown aetiology. The other two diseases are the 'Cadang - Cadang' disease in the Philippines and the 'lethal yellowing' in the north Carribean Islands and the Florida coast.

Spread over nearly 250 thousand hectares of coconut land, the wilt disease is widespread in the southern and central districts of Kerala, where considerable damage is caused to the crop. The spread is not continuous, and pockets of healthy coconut gardens are often seen in the diseased tract. It is found in all soil types - sand, sandy loam, clay as well as laterite. Although the intensity of the disease is more or less the same in hilly laterite as well as in sandy low laying areas, the rate of spread is considerably more in the latter soil type and in the clayey reclaimed and alluvial soils subject to periodical flooding.

The disease is not lethal, quite unlike the Cadang - Cadang or the lethal yellowing. This is a debilitating malady where an affected tree gradually stops yielding nuts in the advanced stages of the disease. However, the leaf rot disease, caused by the fungus Helminthosporium halodes, superimposes itself on the wilt affected trees and together these cause fast deterioration in the general condition and yield.

Although trees of all age groups are susceptible, young trees in the pre-bearing stage and those that have just started bearing succumb to the disease fast. The damage done to the crop at this stage is also the greatest since affected ones in the pre-bearing stage may not flower at all or may do so very late with a poor yield. On the other hand, the yield is not affected much if a tree is diseased after the yield pattern is stabilized (Ramadasan & others, unpublished). Symptoms have not been noticed on seedlings less than four years old.

Flaccidity of leaves is the important primary symptom, often accompanied by marginal necrosis and yellowing of leaflets.

The nature of spread of the disease and the type of symptoms produced suggested that the disease is of pathogenic origin. Varghese (21) originally suggested a virus as a possible cause in 1934. Systematic work by Menon and Nair (6, 7) showed the association of the soil inhabiting fungi Rhizoctonia solani, R. bataticola and Botryodiplodia theobromae with rotting of roots which proved to be a major secondary factor in the disease complex. Since their investigations on the pathogenicity of several bacterial and fungal isolates showed them all to be of secondary importance, they tended to support the earlier theory of a virus origin for the disease.

Studies on the soils showed that in general the coconut soils are poor in nutrients, especially calcium, available potash and total exchangeable bases. Nutrient ratios, particularly K_2O/MgO, K_2O/CaO, N/K_2O, reported by Varghese et al. (20), suggested impaired metabolism.

TRANSMISSION OF THE DISEASE

Coconut

Nagaraj and Menon in 1956 (9) showed that the disease is transmissible by mechanical inoculation of tender shoot of coconut seedlings and young palms with an extract of the diseased leaf tissue in 0.1 M phosphate buffer. Very early symptoms of flaccidity appeared 8 to 12 months after the first inoculation. In this connection, it is interesting to note the method of inoculation reported by Price et al. in 1968 (10) in the transmission of lethal yellowing at Florida. Nagaraj and Menon also reported the transmission of the disease through the banana lace wing bug Stephanitis typicus Distant which feeds and breeds extensively on the leaves of the coconut. Shanta et al. (16) confirmed the results of Nagaraj and Menon on the transmission of the disease in extensive field trials conducted with trees of different age groups (Table 1). It was found that percentage infection of older trees was low and that it was highest in the 6-15 yr age group. No symptoms were seen on seedlings below 4 years old. This corroborates the observations in the field that trees in the flowering stage are the most susceptible.

These inoculation tests for transmission of the disease were, however, conducted in the field in a heavily diseased tract and hence, although indicative, were not conclusive proof of transmissibility. The tests were therefore repeated by Shanta et al. (17) on young healthy coconut seedlings grown in 75 cm tall cement pots of 75 cm diameter, inside an insect-proof screen house. Twenty 2-year old West Coast Tall seedlings obtained from a disease-free area were planted in sterilized soil in these pots. Tender leaf (diseased) extracts in phosphate buffer were smeared on the youngest leaf of 6 seedlings at bi-weekly intervals, new pairs of 6 leaflets being inoculated each time; 200 mesh carborundum was used as abrasive. Controls were inoculated with distilled water. Six seedlings were used for insect transmission tests with adults of S. typicus; 20-25 adults fed for 24 h on diseased coconut leaves, were allowed to feed for about 10 days on a few leaflets of a young opened leaf. Fresh batches of insects held in muslin bags were fed on the same seedling every two weeks. For soil transmission tests, roots from diseased trees, washed and cut into small pieces, were incorporated in the soil at the base of 2 seedlings. The earliest symptom of the disease appeared on one of the 6 seedlings inoculated by the abrasion method 2-1/2 years after the first inoculation. At the end of 3-1/2 years 5 of the 6 seedlings showed some flaccidity of leaflets, stunting and paling of leaves (Table 2). Positive symptoms were obtained on cowpea (Vigna sinensis Endl.) indicator host with 4 of the 5 diseased seedlings. Two seedlings inoculated by S. typicus and one through soil also showed flaccidity of leaflets,

stunting and paling. The soils at the base of all the infected seedlings were found to be infective when tested 4-1/2 years after the inoculations were started. Exact field symptoms were not reproduced in the infected seedlings in this experiment. Nevertheless, the primary symptoms of the disease characterized by flaccidity of leaflets, paling and stunting of leaves were produced. Necrosis of leaves did not develop and the roots did not rot. The roots were later inoculated with R solani which caused considerable damage to the roots. More recent work (3) has shown that marginal necrosis of leaflets which is an associated symptoms of the wilt disease is caused by the secondary pathogen Pestalotia palmarum.

Indicator host

The association of a graft transmissible pathogen with the disease which was transmitted mechanically to the test plant cowpea was reported by Shanta and Menon (14). \A very large number of plants were inoculated by them and about 75% infection was obtained. The symptoms observed were a malformation and 'vein clearing' of the first 1-3 trifoliate leaves when inoculations were done on the 7th or 8th day after sowing. The subsequent leaves were normal, the symptoms produced apparently being a shock-reaction. Closer examination of the inoculated plants showed that the malformation of leaves was due to the necrosis of the cells of the leaf primordia. Similarly, the 'vein clearing' was caused by the failure of development of the parenchyma tissue on the lower surface of the veins. The necrosis of cells was so severe at times that the terminal bud was completely affected in which case the axillary buds often developed into shoots.

Insect transmission

The symptoms on cowpea were also reproduced by forcibly feeding adults of S. typicus fed previously on diseased coconut leaves. About 45% of the adults collected from the field from the coconut palms at Kayamkulam, a heavily diseased tract, were found to be infective. When forcibly fed, the vector required a threshold feeding of 2 h, and a transmission feeding of 18 h. Adults retained infectivity only for 22 to 24 h (19).

Soil transmission

The pathogen is also soil-borne (5, 8). It has been a common observation in the field that when a new seedling is planted after the removal of an older diseased tree, it takes up infection very early. When soil from the base of diseased trees was collected and cowpea sown in it, infection of cowpea seedlings was noticed 10 to 14 days after sowing, the symptoms produced being the same as when it was mechanically inoculated or when fed with infective S. typicus. A number of unknown samples were routinely tested for infectivity by Holmes in 1965 by this method. The symptoms were reproduced when clay and silt fractions of infective soil were separated out and were rubbed on the primary leaves of cowpea. The incorporation of roots of diseased trees, the crushed root extracts or even the root sap

Table 1. Incidence of coconut root (wilt) disease in relation to age.

Treatment	Age in years	Method of inoculation: Mechanical	Insect
Inoculated (I)	2 - 3	0/5*	0/15
Control (C)	"	0/5	0/15
I	4 - 5	7/19	0/10
C	"	1/14	0/9
I	6 -10	5/11	4/18
C	"	0/11	1/17
I	10 -15	10/17	9/19
C	"	2/16	2/17
I	20 -30	2/5	not tested
C	"	0/5	" "
I	35 -45	not tested	2/6
C	"	" "	0/6
I	40 -50	0/12	10/22
C	"	0/6	1/22

* Denominator indicates number of palms inoculated and nominator the number showing symptoms

Table 2. Results of transmission trials on coconut seedlings conducted inside the insect-proof screen house (Inoculations started in July, 1959)

Treatment	Seedling No.	Condition of seedling in 1960 June	1961 Jan.	1961 June	1962 Jan.	1962 June
Sap inoculation	1	H	H(d)	H(d)	DE	DE
	2	H	H	H	H	H(d)
	3	H(d)	H(d)	H(d)	DE	DE
	4	H	H(d)	H(d)	H(d)	DE
	5	H	H(d)	H(d)	DE	DE
	6	H	H(d)	DE	DE	DE
Insect transmission	1	H	H	H	H(d)	H(d)
	2	H	H	H(d)	H(d)	H(d)
	3	H	H	H	H(d)	H(d)
	4	H	H	DE	DE	DE
	5	H	H	H(d)	H(d)	DE
	6	H	H	H	H(d)	H(d)
Control	1	H	H	H	H	H
	2	H	H	H	H	H

H : Healthy; H(d) : Palms with doubtful symptoms of flaccidity and paling;
DE : Early stage of disease

Table 3. Disease incidence on cowpea when inoculated with different samples.

Method of inoculation	No. of seedlings inoculated or sown	% infection
Cowpea sown *in situ* in infective soil from field	228	32
Sterilized soil watered with infective leaf extract	63	84
Sterilized soil watered with root extract	57	76
Sterilized soil watered with soil suspension	108	44
Incorporation of artificially infected plant parts into sterilized soil	48	70
Naturally infected soil with roots	49	65
Naturally infected soil without roots	98	17
Clay & silt fraction of infective soil (mechanical inoculation)	216	48

Table 4. Presence or absence of the pathogen in different parts of inoculated cowpea seedlings

Incubation period in h	Source of inoculum				
	Simple leaves	Growing shoot apex	Stem up to Cotyledons	Stem below Cotyledons	Root
24	0/16	0/16	0/16	0/16	0/16
48	4/24	0/16	0/16	0/16	0/16
72	9/28	7/16	2/21	0/16	0/16
96	13/29	13/24	11/22	7/13	6/15

Denominator indicates the number of plants inoculated and nominator the number infected.

from diseased trees in sterilized soil where seeds were sown, produced infection on cowpea (Table 3). Sterilized soil in pots where artificially infected cowpea was grown for about 6 weeks or more was found to have become infective. Infectivity was enhanced when the roots and also the entire plants were finely chopped and incorporated in the soil. Leachates from diseased soil were also infective. The soils from the base of potted coconut seedlings used for transmission trials in the insect-proof screen house were also found to be infective after the symptoms were apparent, for a year or two. Apparently, the pathogen is released through roots. Soil samples from a highly diseased coconut garden from where all trees were removed, were found to be still infective 28 months after removal of the palms. This also indicates the comparatively high stability of the pathogen under natural conditions (13).

Transmission into other palms

The pathogen was also transmitted artificially to seedlings of the common arecanut, Areca catechu Linn. (11). Mechanical inoculation by the abrasion method was done with crude infective sap from coconut leaves every fourth month. Sixteen months after the first inoculation, 6 of the 12 inoculated seedlings developed severe necrosis along veins of the youngest one or two leaves. The pathogen was detected in these seedlings when indexed on cowpea. The subsequent leaves appeared normal, the symptoms apparently being only a shock-reaction as in the case of cowpea. The oil palm (Elaeis guineensis Jacq.) on the other hand, at least in the preliminary trials, indicated that like coconut it is a systemic host of the pathogen (12). No symptoms were observed on 2 year old seedlings when inoculated artificially. However, when flaccidity of leaflets similar to that observed in coconut was noticed on some oil palms growing at the Research Institute at Kayamkulam, they were indexed on cowpea. Twenty-nine of the 59 palms tested were seen to be infected. It was also observed that a large number of them got diseased at or about the commencement of bearing.

PROPERTIES OF THE PATHOGEN

Studies on the physical properties of the pathogen in leaf extracts of diseased coconut indicated a thermal death point of 76°C and a dilution end point of 10^{-4}. It was most effective at pH 5.0 to 9.0 in supernatant solutions of the extract. When stored frozen, it was active for 9 weeks and at room temperature for 3 weeks in extracted sap (15). Studies on movement of the pathogen in inoculated cowpea seedlings showed that it moved down to the roots within 96 h after inoculation of primary leaves (Table 4). Following this, it was observed that the symptoms are graft transmissible only if grafts were made between cowpea seedlings within a week after inoculation.

Considering the modes of transmission and the properties of the pathogen, preliminary tests were made at Kayamkulam for transmission of tobacco mosaic virus and tobacco necrosis virus, but without success. Judging from the peculiar nature of symptoms on cowpea and lack of evidence on filterability, Holmes (4) sug-

gested that this might be a spirochaete or a virus-like pathogen.

PURIFICATION

High concentration of tannins in the coconut tissues was found to interefere considerably with the purification of the pathogen. Recently, Summanwar et al. (18) reported the purification and isolation from diseased coconut material of a rod-shaped virus (320-360 mμ x 24-25 mμ) which produced local lesions on Chenopodium amaranticolor from which it was passed on to Nicotiana glutinosa and other test plants. The method of purification used by them was extraction in distilled water followed by 2 cycles of differential centrifugation. However, repeated bioassay tests with extracts from diseased coconut leaves and roots at Kayamkulam, purified by the same method, failed to produce any local lesions on C. amaranticolor or N. glutinosa.

Further wokr is being done to standardize a method of purification and to see exactly how the tannins interfere in the purification work.

COWPEA TEST

Recently cowpea has ceased to respond to inoculation, both mechanical as well as by S. typicus. Studies with different buffers and the incorporation of different chemicals commonly used in virus extraction, in the extracting solutions failed to infect cowpea (1, 2). Studies on the environmental factors that might influence susceptibility of cowpea showed that sudden changes in the environment, especially temperature, had a profound influence on susceptibility of cowpea. Yet, it is not clear how a host plant which was once so uniformly susceptible to the pathogen should have lost this property now.

An extensive search for a suitable test plant among the local weeds, both monocots and dicots, is in progress, but so far it has been unsuccessful.

DISCUSSION

It may be seen from the data presented above that an infectious agent causes a systemic, apparently mild, disease on coconut which now seems to be manifested by mild or severe flaccidity of leaves. This by itself does not apparently cause much damage to the crop. However, coconut being a perennial plantation crop with a really long life span of 80 to 100 yr is liable to be attacked by many soil inhabitants which may not get a chance to invade the ordinary seasonal crops in the normal course of events. All defects of monoculture are likely to appear in this crop. Thus, a natural assumption resulting from the present investigations on the wilt disease is that in the wake of the primary pathogen, by itslef mild perhaps, all secondary organisms, soil-borne as well as air-borne, find a way into the tissues of the palm, the result being a devastating, complex disease.

The soils of Kerala are well-known for their deficiency of calcium and potassium. An induced deficiency of magnesium and the abnormal leaching of nutrients by the heavy monsoon waters

result in severe yellowing of leaves throughout the coastal sandy tracts. Yellowing thus naturally becomes a part of the wilt disease complex.

The problem on hand is the identification of the pathogen and the standardizationof a technique for its isolation and easy diagnosis in soils and tissues. So long as cowpea was showing symptoms, the type of symptom produced or the nature of the pathogen causing this were not of immediate concern. But now it has become imperative that a method is devised for the detection of the pathogen. When the virus isolated by Summanwar et al. (18), is finally confirmed as the causal agent by pathogenicity trials and/or by its constant association with the diseased trees alone, a major step towards solving the aetiology of the disease could be considered as accomplished. Concerted efforts are made towards that end now.

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