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Additional evidence of soil transmission of coconut root (wilt) pathogen

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ABSTRACT

A sap-transmissible pathogen resembling a virus is associated with the root (wilt) disease of coconut. This pathogen is soil borne. Cowpea plants became diseased when grown in infective soil, or in sterilized soil watered with infective leaf or root sap, or in soil to which infected roots were added. Treatment with pentachloronitrobenzene destroyed infectivity. Air-drying for more than a week or fine grinding do not destroy infectivity. The pathogen perhaps is released through roots of infected plants. Soil water plays a major part in spread of the disease in nature.

The root (wilt) disease of coconut in Kerala is the cause of heavy loss to the economy of this important cash crop. It occurs in the southern and central districts of Kerala and is slowly spreading both toward north and south. It is a debilitating, systemic disease. The major symptom of infected trees in the field is flaccidity of leaflets. In older palms other symptoms like yellowing of outer whorl of leaves, marginal necrosis of leaflets and petiole bending and breaking also occur singly or in combination. The disease is found in all soil types although it is more severe and spreads faster in low lying areas and along river banks subject to periodical inundation.

The cause of the disease is considered to be a mechanically transmissible pathogen resembling a virus—the identity of which is as yet to be established. Transmission trials by mechanical inoculation produced characteristic flaccidity of leaflets on coconut seedlings both in the field and under controlled conditions (Nagaraj and Menon, 1956; Shanta *et al.*, 1964). The pathogen is also soil borne. Summanwar *et al.* (1969) reported the presence of rod shaped particles (320–360 mμ × 24–25 mμ) in extracts of diseased coconut roots and leaves.

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MATERIALS AND METHODS

Cowpea (*Vigna sinensis* Endl. var. 'New Era.) seedlings were used throughout these investigations as the indicator host (Shanta and Menon, 1960; Holmes *et al.*, 1965) and it was considered to be infected when symptoms as described by Shanta and Menon were apparent. Infectivity of soil samples was tested by mechanically inoculating, clay and silt fractions (Menon and Shanta, 1962) on primary leaves of cowpea.

RESULTS AND DISCUSSION

A number of small experiments were done to study the infectivity of soil (Table 1). Cowpea seeds sown in the soil collected from the base of diseased trees gave 35 to 40 per cent infection of emerged seedlings. Cowpea seedlings also became infected when grown in sterilized soil watered with infective leaf or root sap of naturally infected coconut trees. The incorporation of roots and leaves of artificially infected cowpea seedlings into sterilized soil made it infective. Presence of large number of diseased roots in soil increased disease incidence in cowpea. About 50–60 per cent plants were infected when clay and silt fractions of infective

Table 1. Disease incidence on cowpea when inoculated with different samples

Method of inoculation	Percentage infection
Cowpea sown *in situ* in infective soil from field	32
Sterilized soil watered with infective leaf sap	84
Sterilized soil watered with infective root sap	76
Sterilized soil watered with infective soil suspension	44
Incorporation of artificially infected plant parts into sterilized soil	70
Naturally infected soil with roots	65
Naturally infected soil without any roots	17
Clay and silt fraction of infective soil (Mechanical inoculation)	48

soils were inoculated on leaves of cowpea mechanically. The above results clearly indicate that cowpea plants get infected through roots and that infected plant debris enhances the infectivity of soil. The evidence that the infective principle is released through roots into soil was obtained when sterilized soil in which infected copwea seedlings were growing became infective 4 to 6 weeks after inoculation of the seedlings. This was corroborated by transmission trials on coconut seedlings. It was found that the sterilized soils in which these plants were growing became infective 1 to 3 years after the plants became diseased.

The infectivity of soils at different depths and distances from the base of diseased trees was studied. It was found that soil to a depth of 1 metre and up to a distance of 4 metres from the base of diseased trees were infective. Maximum infectivity was noticed in the region of actively growing roots. Preliminary studies were conducted to find out the survival of the pathogen in soil when stored under wet and dry conditions at room temperature. All visible particles of roots and debris were removed from the originally infective soil which was passed through a 20 mesh sieve. Part of the soil was stored dry whereas the other was kept saturated with water. It was found that infectivity was retained in all samples up to 12 to 20 days. One sample of soil was infective under both conditions of storage up to 40 days.

In attempts to study the effect of some insecticides, fungicides and nematicides on infectivity of soil, samples of naturally infected, air-dried soil were treated with different concentrations of pentachloronitrobenzene (PCNB), flit 406, nemagon, chlordane, aldrin and heptachlor. Infectivity of the treated samples were tested by growing cowpea seedlings *in situ*, one week after treatment. Only PCNB was found to be effective in disinfecting the soil (Table 2). Nemagon was phytotoxic even at 1 ml per kg soil, the lowest concentration tested. Drenching infected soil with a concentrated solution of calcium hydroxide was also found to inhibit infectivity of soil (Holmes *et al.*, 1965). Observations recorded in experiments conducted to study the effect of surface sterilizing the roots cowpea of seedlings raised in infective soil with 0·1 per cent mercuric chloride before transplantation into sterilized garden soil show that mercuric chloride treatment interferes with the early phase of infection. Grinding the soil finely did not destroy infectivity.

In preliminary experiments conducted to study movements of pathogen in relation to flow of water, long rectangular wooden trays were filled one half each with sterilized and unsterilized infective soil. Cowpea seeds were sown in the two halves and soil was watered with sterilized water, taking care that no direct mixing of soil occurred. In those trays where leachates from infective soil flowed to sterilized soil, the seedlings in the latter also became diseased within 10 days of sowing. In the others where flow of leachates were in the opposite direction and in those where apparent stagnation occurred a small number of seedlings in the sterilised soil became diseased 4 to 6 weeks after soing (Table 3).

Observations on the spread of disease over a three year period in different soil types in the field showed rapid and indiscriminate spread in reclaimed sandy soil with a water-table about 30·5 cm from the surface. Fairly rapid spread was

Table 2. Effect of chemicals on infectivity of soil (total number of samples tested is 8)

Chemical tested	Concentration used per kg soil	No. of samples infective/Number tested			
		4 days	7 days	14 days	21 days
PCNB	2·5 g	-/8	-/8	-/8	-/4
	5·0 g	-/8	-/8	-/8	-/4
Flit 406	2·5 g	-/8	2/8	2/8	2/4
	5·0 g	-/8	4/8	6/8	1/4
Nemagon 72% EC	1·0 ml	*Toxic	1/8	4/8	4/4
	2·5 ml	*Toxic	Toxic	2/8	4/4
	5·0 ml	Toxic-no germination			
	10·0 ml	Toxic-no germination			
Chlordane 10% dust	2·5 g	5/8	2/8	2/8	-/4
	5·0 g	5/8	2/8	2/8	-/4
	10·0 g	2/8	-/8	3/8	-/4
Aldrin 5% dust	2·5 g	4/8	2/8	3/8	-/4
	5·0 g	5/8	2/8	1/8	-/4
	10·0 g	1/8	2/8	-/8	-/4
Heptachlor 3% dust	2·5 g	1/8	1/8	4/8	-/4
	5·0 g	1/8	1/8	4/8	-/4
	10·0 g	3/8	2/8	2/8	-/4
Untreated control		7/8	8/8	8/8	4/4

*Stunted seedlings with yellow leaves. At the two higher concentrations there was no germination.

observed in sandy soil with a water-table about 91 to 182 cm from the surface. Similar field observations show high incidence of disease along river banks and other water ways or in areas with high water-table. The occurrence of the disease in patches in the diseased belt further confirms the soil-borne nature of the pathogen.

The evidence presented above confirms the previous findings of Menon and Shanta (1962) and Holmes *et al.* (1965) that the coconut wilt pathogen is soil transmitted. Comparison of its method of transmission with that of other viruses reveals some interesting features. The two major groups of biological vectors so far recognised, aiding virus transmission in soil are the nematodes and the chitrid fungi. A biological vector does not seem to be essential for the transmission of the coconut wilt pathogen since addition of leaf extract or roots of artificially infected plants into sterilized soil make the latter infective. That nematodes are not directly involved in transmission is perhaps also indicated by the fact that infectivity survives more than one week's air-drying and that it is not lost by fine grinding. The presence of species of *Xiphenema* and *Longidorus* in all soil types, the former both in diseased and healthy tracts, the latter only in diseased areas or very near the border between the two would be of importance. These and other nematodes might play an indirect role by acting as disease incitants

Table 3. Disease incidence in cowpea in relation to flow of water

Direction of water flow	Sterilized soil		Infective soil	
	Percentage infection in cowpea			
	10 days	30 days	10 days	30 days
Infective to sterilized soil	20	33	30	36
Sterilized to infective soil		12	10	28
Static		10	22	25

(Weischer, 1967). Although biological vector is seemingly not necessary for transmission, the effect of chemicals such as PCNB and mercuric chloride would seem to indicate the presence of such an organism. In this context the behaviour of tobacco necrosis virus in soil may be considered. Two kinds of transmission are suggested for this virus, one fungus-dependant and the other not (Babos and Kassanis, 1963). No evidence has been obtained so far as to the particular role of fungi in the transmission of the wilt pathogen. However, it seems probable that, as in the case of TNV, soil water is important for its dessemination. It may be that either fine particles of infective clay or fungal zoospores that act as vectors are carried about by water. That virus occurs adsorbed on clay particles is reported from Japan where wheat and barley yellow mosaic viruses are soil-borne (Miyamoto, 1959). Perhaps the best example for the phenomenon of virus adsorption on clay particles is provided by tobacco mosaic virus which over-winters in infected plant debris and as the virus is leached out from the debris is adsorbed by clays (van der Want, 1952). In this connection the observation of TMV in purified preparations from diseased coconut roots by Summanwar *et al.* (1969) is of interest.

The importance of water in relation to the occurrence of root (wilt) disease is highly evident but its exact role in the incidence and spread of disease remains to be studied.

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*Originals not seen.