

## STUDIES ON "LEAF SCORCH DECLINE"—OF COCONUT

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### SUMMARY

"Leaf Scorch Decline" of Coconut in Sri Lanka has remained a disorder of unknown etiology for a long time. The present study was initiated in order to determine its possible cause.

An estimation of the "soil" and "rhizosphere" bacteria has been made by the dilution plate technique.

The results indicate a constant association of *pseudomonad* type of bacteria in the soil around diseased palms and in necrotic roots and other plant parts of diseased palms. This appears to suggest the possibility of one or more of these bacteria either alone or in combination with other organisms and/or factors as a possible causal agency. Susceptibility to the disorder would appear to be contingent on reduction in vigour and health of the palms involved.

### INTRODUCTION

"Leaf scorch decline" of coconut is a disorder first recorded in Sri Lanka in 1955 (Kirthisinghe, 1966) and is prevalent in the Southern Province especially in Gonapinuwala, Buddagama and Elpitiya areas. Incidence of this disorder has also been observed in the main coconut growing area, the North Western Province of Sri Lanka. Symptoms of the disease have been recorded from time to time only on bearing adult palms over 20 years of age (Salgado, 1960; Abeygoonawardene, 1962; Davies, 1962; Ekanayake, 1963; Peries and Kirthisinghe, 1967; Peries, 1968; Kranz, 1967 & Humphries, 1970).

Visual symptoms are the necrosis ("scorch") in the leaflet of the mature frond starting from the tip, the dieback (decay) of the root system where the intensity is proportional to the extent of decline, tapering of the trunk in the crown region towards the last stages of the disorder accompanied by a reduction in the number and size of the nuts.

Investigations carried out to establish the cause of the disorder have been reviewed chronologically by Kirthisinghe (1966). There is no evidence that the disorder is caused by the deficiency of major or minor nutrient elements (Nethsinghe, 1966; Balakrishnamurthi, 1969). The possibility of nematodes being the causal agent has been ruled out by Robertson (1965), and Kranz (1967) ruled out the possible involvement of foliar pathogens.

Increase in *Fusarium* and *Cylindrocarpon* (Kranz, 1967) and *Fusarium* (Peries, 1968) populations in soil around palms showing signs of severe leaf scorch as compared with soil around healthy palms, and the association of these fungi with decaying necrotic root systems of

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diseased palms has been recorded. Root inoculations by Peries (1968) with *Fusarium* sp. isolated from the roots and the soil around diseased palms showed that none of the species is pathogenic.

Virological studies, carried out by Humphries (1971) on specimens provided by the CRI, has ruled out viruses as the causal agent of the disorder. In view of the lethality of the disease and the uncertain nature of the causal agent the present studies were undertaken in order to determine the possible cause of the disorder.

### MATERIAL AND METHODS

Soil and root samples were taken from four points about one meter away from the bole, after removing the top soil, in the form of soil blocks 15 x 15 x 30 cm in dimension. These were brought to the laboratory in sterile plastic boxes. A composite sample was made by mixing the four samples after carefully removing all root pieces in the soil blocks. The composite sample constitutes what is referred to as the 'field soil'.

The 'rhizosphere soil' that is used was obtained in the following manner. Eight root pieces, each 2.5—4.0 cm in length were placed in a sterile 250 ml conical flask and shaken at full speed for 5 minutes in a Griffin flask shaker to remove clumps of soil adhering to the root pieces. The root pieces were then placed in 75 ml sterile distilled water contained in a 250 ml conical flask and shaken again for 5 minutes to yield a rhizosphere soil solution. After filtering and air drying the weight of the 'rhizosphere soil' was obtained.

The estimation of the soil and rhizosphere bacteria was made by the dilution plate technique. Two grammes of air dry soil was mechanically shaken for 10 minutes with 50 ml of sterile distilled water and a dilution series giving  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$  dilutions were prepared in the usual manner. When 'rhizosphere soil' is to be used the above dilution series were prepared using the rhizosphere soil solution.

For bacterial population estimations 0.5 ml of the  $10^{-3}$  dilution was added to the surface of 20 ml Oxoid nutrient agar CM3 contained in a petri dish. The solution was spread over the surface with a bent glass rod, dried at 40°C and incubated at 37°C for 24 hours. Bacteria were also isolated from leaflets, petioles and various portions of the stem and root. Samples were thoroughly washed and cut into 2 cm portions and the surface sterilised using 0.1%  $\text{HgCl}_2$ . These were then washed in five changes of sterile distilled water and the centre portions of roots and petioles crushed and made into a suspension in a small amount of sterile water. A loopful of the suspension with the debris was streaked on plates of nutrient agar CM3 and incubated at 37°C for 24 hrs.

### RESULTS

#### Bacterial populations in the 'field soil' and 'rhizosphere soil'

Results of the bacterial population estimations, given in Table 1, show an increase in the total bacterial population in the 'field soil' obtained from the root zones of moderately and severely diseased palms when compared with the 'field soil' obtained from the root zones of healthy palms. The total bacterial population remains more or less constant in the rhizosphere of the roots from healthy, moderately and severely diseased palms. But the bacterial population of the rhizosphere show a 6—10 fold increase when compared with that of the 'field soil'. However, a sharp increase in the population of a gram-ve rod shaped bacterium (type 1 in Table 1) was observed in the rhizosphere of moderately and advanced diseased roots when compared with the rhizosphere of healthy roots.

Table 1. Total bacterial population estimations in the 'field soil' and the 'rhizosphere soil' of healthy, moderately and severely diseased palms.

		'Field soil'		'Rhizosphere soil'	
		Total bacteria /g of soil	Total bacteria /g of soil	Total bacteria /g of soil	Type 1* /g of soil
Healthy	{ 1	2.8 x 10 <sup>6</sup>	29.0 x 10 <sup>6</sup>	—	—
	{ 2	2.0 x 10 <sup>6</sup>	20.0 x 10 <sup>6</sup>	4.2 x 10 <sup>6</sup>	—
Moderately diseased	{ 1	—	—	—	—
	{ 2	3.0 x 10 <sup>6</sup>	23.0 x 10 <sup>6</sup>	6.6 x 10 <sup>6</sup>	—
Severely diseased	{ 1	3.6 x 10 <sup>6</sup>	28.5 x 10 <sup>6</sup>	—	—
	{ 2	3.5 x 10 <sup>6</sup>	20.0 x 10 <sup>6</sup>	7.7 x 10 <sup>6</sup>	—

\* gram - ve, rod shaped, motile bacteria with glistening colonies on Oxoid nutrient agar CM3.

#### Bacterial population inside diseased palms

Isolations of bacteria were made from inside of root samples obtained from soil blocks. Healthy, moderately and severely diseased palms all yielded bacteria from inside their roots. However, only 8% of healthy roots yielded bacteria when compared with the yield of 44% and 56%, respectively, from moderately and severely diseased roots.

The inside of stems, leaves and petioles of only diseased palms yielded bacteria. A total of 26 isolates (21 from diseased palms, 1 from the rhizosphere of diseased roots (type 1) and 4 from the healthy roots) were characterised using the determinative tests of Lelliott *et al.* (1966). The results in Table 2 show that all except the 4 isolates from the roots of healthy palms fall into four fluorescent pathogenic *pseudomonad* groups. The four isolates from healthy roots were gram +ve and did not show any fluorescence. They may be considered to fall into non-pathogenic group V of Lelliott *et al.* (1966).

#### Pathogenicity tests

Inoculations were carried out to establish whether the bacteria of the four groups, either alone or in combination are pathogenic to coconut palms. Experiments using leaflets whose cut ends were immersed in 1/8 strength nutrient broth cultures of the bacteria did not yield any conclusive results. Although necrotic (scorch) symptoms appeared on some of the leaflets in inoculated cultures in about 48 hours, controls also showed similar symptoms a day or two later. Addition of bacterial cultures to the root systems of seedlings and young palms also failed to produce symptoms. Bacterial suspensions in 1/8 strength nutrient broth when injected on to the trunks also failed to produce symptoms.

#### DISCUSSION

Results of the present investigation indicate a preponderance of rod shaped bacteria of *pseudomonad* type in the soil around diseased palms and in the rhizosphere of diseased roots. Although these increases may be due to the abundance of dead root matter caused by the activity of some other organism, eg. *Fusarium* as suggested by Peries (1968), the isolation of *pseudomonad* type of bacteria in considerable numbers from stems, petioles and leaflets of diseased palms suggest a more positive role for these bacteria.

Table 2. Characteristics and groupings of the bacterial isolates using the determinative tests of Lelliott, Billing and Hayward (1966).

<i>number of isolates</i>	<i>gram reaction</i>	<i>type</i>	<i>fluorescence</i>	<i>levan</i>	<i>oxidase</i>	<i>potato rot</i>	<i>nitrate reduction</i>	<i>acid from sucrose</i>	<i>lipase</i>	<i>Group, (Lelliott et. al. (1966))</i>
5*	-	motile rod	+	+	-	-	-	+	-	Ia
9	-	motile rod	+	-	-	-	-	+	-	Ib
4	-	motile rod	-	+	+	+	+	÷	+/-	IVa
4	+	motile rod	+	-	+	+	-	+/-	÷/-	IVb
4**	+	motile rod	-	-	+	-	+/-	+/-	÷	V?

\* include isolate (type 1) from the rhizosphere

\*\* isolates from the healthy roots

The consistent association of these pseudomonad type of bacteria with the disease in the soil, in the necrotic roots and in other parts of diseased palms suggest that it is caused by one or more of these bacteria either alone or in combination with other causal organisms and or factors.

The failure to introduce the disease by artificial inoculations with bacteria in the present studies and with *Fusarium* in the studies of Peries (1968), suggest the probable involvement of predisposing factors such as the weakening of the palms by adverse soil conditions and/or other factors which reduce their vigour. This predisposition seems to be a primary requirement as the disease is always observed in palms over about 20 years of age and never in young palms.

Occurrence of the disease in an isolated area near the kaolin dumps of the Ceramic Factory, Negombo, gives weight to this suggestion; that adverse soil conditions and/or other factors predispose the healthy palms to the disease. On the other hand, a change in the soil conditions may cause an abnormal increase in bacterial and/or fungal population in the 'field soil' and the 'rhizosphere soil', and as suggested by Humphries (1970) might produce toxins which when absorbed and translocated to the leaves cause the characteristic symptoms. Once the disease is initiated the bacteria and/or fungi would enter the tissues whose vigour would have been reduced by the toxins that were absorbed.

Further work along these lines would be of value since it was observed that the affected palms lose their economic value due to a reduction in the output of copra and oil from their nuts (unpublished data) after 2—3 years of the appearance of the symptoms.

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