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STUDIES ON LEAF SCORCH DECLINE OF COCONUT PALMS

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INTRODUCTION

Maramorosch (1964) has recently described the diseases of unknown etiology which affect coconut palms, growing in different parts of the world ; " Leaf Scorch Decline " occurring in Ceylon is one such disease. The symptoms of these diseases are generally distinct, but Davis (1962) has compared the disease in Ceylon with " Root Wilt " in India and suggested that Leaf Scorch Decline had its origin in India.

Leaf Scorch Decline was first recorded in Ceylon in 1955 (Kirthisinghe, 1966), but it is quite likely that palms were affected many years before reports were made to the Coconut Research Institute. The disease is most prevalent up to date in the Galle District, where it was first recorded.

The symptoms of the disease have been described briefly from time to time (Salgado, 1960 ; Abeygunawardena, 1962 ; Davis, 1962 ; Ekanayake, 1963 and Peries and Kirthisinghe, 1967). The most characteristic visual symptom of the disease, from which it gets its name, is a necrosis (scorch) on the lower mature whorl of fronds of trees, generally above 20 years of age. When the root system of affected trees is exposed, their rootlets are found to be decayed, the intensity of decay increasing with that of Leaf Scorch. This is accompanied by a tapering of the trunk and a reduction of the size of the crown, including inflorescences and nuts. In the final stages of the disease, the crown becomes reduced to a tuft of small leaves, without inflorescences, and this eventually falls off. The full course of the disease may take 2-6 years. However, it is important to note that this is a lethal disease, unlike some of the similar diseases described by Maramorosch (1964).

Preliminary investigations, carried out to establish the nature of the disease, have been reported chronologically by Kirthisinghe (1966). *Ad hoc* experiments carried out by the Coconut Research Institute, in attempts to control the disease by the application of fertilizers and fungicides, both to the soil and foliage, have not given conclusive results. It has been reported, however, that the disease is not caused by a nematode infection (Robertson, 1965). There is also some evidence to indicate that the scorch is not caused by a foliar pathogen (Kranz, 1967) ; but the cause of the disease is not yet known.

As the disease is eventually lethal, it is clear that its rapid spread in the areas North of Colombo, which are the most important coconut growing areas in the country, would be of vital economic importance to this country. The disease runs its full course over a period of 2-6 years. Therefore, it is possible that many apparently healthy palms in some districts may in fact be affected by the disease. The effect of incipient disease on the yield of palms, although not established, could be quite significant. The present studies were undertaken in order to establish the cause of the disease and to devise control measures against it.

MATERIALS AND METHODS

Materials :

Uniform soil samples were collected from four points round the base of healthy and diseased palms, at a distance of three feet from the bole of each, and a depth of 3 ins. The four samples from each palm were placed in a single polythene bag, mixed thoroughly in the field, and brought back to the laboratory for investigation.

Parts of the fronds, inflorescences, roots and trunk of healthy and diseased palms were collected by cutting down representative trees and sampling with a saw, a knife or chisel as necessary. The different samples for laboratory investigations were brought back in polythene bags.

Methods :

The comparative studies on the distribution of soil microflora were carried out by an adaptation of the micro-sieve technique described by Parkinson and Williams (1961). This gave a comparatively broader representation of the fungi in the soil than the soil plate method (Warcup, 1950) and the hyphal isolation method (Warcup, 1955).

According to the technique adopted, 1 kg. of field soil was air dried overnight in the laboratory, and passed through a 2 mm. sieve to eliminate stones, roots and other large particles. 0.5 gm. of the sieved soil was thoroughly soaked in about 25 ml. of sterile tap water, until the soil crumbs were easily broken. This soil was carefully washed on to a sieve (B.S. 325 ; 30-45/u) soldered to the base (2″ × 2″) of a metal box 3½″ high. A jet of sterile tap water under a constant pressure of 1-2 lb./in 2, from an aspirator bottle, was then made to play upon and agitate the soil on the sieve. 10 litres of water was used for this washing. In this manner, fungal spores and the clay fraction were washed away, leaving the sand, humus and hyphal fragments. The residue left on the sieve was washed into a measuring cylinder and the volume of water brought up to 100 ml. This suspension was agitated to obtain an even spread of particles, and 0.5 ml. of it was drawn out and placed in a sterile petri dish. Molten Czepak-Dox + Yeast agar at about 40°C was then poured into each plate and mixed thoroughly with the soil suspension to give an even spread of the particles. Plates were incubated at room temperature and, after 12 hrs., examined under a low power (× 50) microscope for any signs of fungal growth. Growing colonies were picked out and transferred into agar slopes in tubes. This was continued for three days, until all colonies were isolated.

Material for histological studies was cut into small pieces of suitable size, fixed in F.A.A. and embedded in wax according to the method described by Johanson (1950). Sections, 10-15/u thick, were cut with a sledge-type microtome and stained by Cartwright's (1930) double stain technique, which gave satisfactory differentiation. Free hand sections too, stained with cotton blue, were examined at the same time.

Portions of leaves and roots, from the leading edges of necrotic patches, were washed for two hours in running water, surface sterilized by rapid immersion in 0.1% mercuric chloride, then washed in three changes of sterile distilled water and plated on Difco Potato Dextrose Agar (P.D.A.).

RESULTS

Soil Microbiology :

The microbiological examination of the soil showed a significant difference between the distribution of fungi in the root zone of healthy and diseased palms. Whereas there was a variety of fungi belonging to different species of various genera in the soil at the base of healthy palms,

the fungal population in the soil associated with diseased palms consisted predominantly of two or three species of *Fusarium*. Table I gives the complete details regarding the fungi isolated from the two types of soils, along with the approximate frequency distribution of each species, expressed as a percentage of the total number of colonies present in each soil. This Table shows that approximately 60% of the fungi isolated from the soil around diseased palms belongs to the genus *Fusarium*. This abnormality in the distribution of fungal flora was quite characteristic and was associated with the soil in the root zone of all diseased palms examined. This study was repeated twice, giving very similar results.

TABLE I

The distribution of soil fungi in the root zones of healthy and diseased palms, expressed as a percentage of the total numbers of fungi isolated from each

Fungus	*Healthy*	*Leaf Scorch Decline*
Aspergillus spp.	6	3
Botryodiplodia theobromae ...	11	9
Curvularia spp.	1	—
Cylindrocarpon spp.	<1	—
Fusarium spp.	14	60
Mucor spp.	4	1
Nigrospora spp.	2	—
Penicillium spp.	11	5
Phytophthora spp.	1	—
Rhixoctonia spp.	3	8
Rhizopus spp.	7	—
Sterile mycelia	20	9
Trichoderma viride	12	—
Unidentified species	8	5

< Less than

Root System :

The roots of healthy and diseased palms were exposed by using a powerful jet of water from a pressure pump, to clear the soil. The root system of diseased palms appeared to be more fibrous than that of healthy ones. Close examination showed that the root system of diseased trees became excessively fibrous because of the decay of young rootlets. Immature laterals were found to decay after growing about 2-3 cm., this resulted in the decayed root producing a new lateral, which rots in turn, giving rise to a further lateral which suffers the same fate. This occurs repeatedly, giving rise to a close network of roots. Goodey (1964) has reported a similar type of root rot in association with the disease.

The decay of the root tips is apparently caused by a pathogen. Preliminary studies to establish the identity of the causal organism have indicated that the root necrosis may be caused by a *Fusarium* spp., which was isolated from such roots on several occasions. The results obtained from 125 isolations, made from decayed and healthy root tips, are presented in Table 2. Approximately 50% of the fungi isolated from decayed root tips belonged to the species *Fusarium*, as compared to only 15% from healthy roots.

TABLE 2

Nos. of fungi isolated from decayed and healthy root tips expressed as a percentage of the totals isolated

Fungus	*Healthy roots*	*Decayed roots*
Aspergillus spp.	5	7
Botryodiplodia thiobromae ...	10	12
Cylindrocarpon spp. ...	2	—
Cylindrocladium spp.	5	—
Fusarium spp.	15	50
Mucor spp.	10	2
Penicillium spp.	10	12
Rhizoctonia spp.	15	—
Sterile mycelia	8	7
Trichoderma viride	12	—
Unidentified species	8	10

Innoculation experiments were carried out to establish whether the three most common species of *Fusarium*, isolated from decayed roots, were pathogenic to coconut rootlets. Two types of innoculations were carried out : in the first the fungi, growing on P.D.A., was placed under small flaps of the epidermis of young roots in 1-year old seedlings, the complete procedure being carried out under aseptic conditions. In the second method, the contents of ten 10-day old petri dish cultures of the fungi, growing on P.D.A., were mixed with 500 gm. of soil, and 100 gm. portions of this soil-culture mixture was bound on to young coconut roots with a muslin and surgical tape. The results were recorded 4 and 10 days after pure and soil mixture inoculations, respectively. None of the trials gave positive results, most of the direct inoculations being overgrown with contaminants in a few days. A slight blackening of the superficial layers of the rootlets had occurred in several cases with the soil inoculation technique. However, further examination showed that the fungus had not penetrated the root tissue in this area.

Histological studies

There was a fairly clear difference, obvious to the naked eye, between the appearance of the cross section of the trunk of healthy and diseased palms. In the present studies, the centre of the section of the trunk of diseased palms was obviously much darker brown than that of healthy palms. This observation needs confirmation in view of the reports made by Ekanayake (1963), who did not observe any discolouration or anatomical abnormalities in longitudinal (L.S.) and transverse (T.S.) of the trunks of diseased palms.

The most characteristic feature seen during the microscopic examination of thin sections, both L.S. and T.S., was the damage to the vascular tissue in all parts : roots, trunk and fronds of diseased palms as compared to healthy ones. The xylem vessels of diseased palms were clogged with tyloses, where as few tyloses were observed in the tissue of healthy palms. It is quite clear that the conduction of water and nutrients would be considerably hampered by the tyloses present in diseased palms.

Esau (1965) has discussed the significance of tyloses and the factors that lead to their formation in vascular plants. Tyloses can be produced following virus infection, mechanical injury and fungal or nematode infection of plants. Excessive tylose formation has been observed in

A palm affected by "Leaf Scorch Decline"

rubber trees infected with the bark cracking virus (Peries and Satchuthanandavale, 1964) and by *Fomes lignosus*, the White Root disease fungus (Peries, unpublished data). It is clearly necessary to investigate the factors which lead to tylose formation in coconut palms affected by Leaf Scorch Decline, as they appear to be closely linked with the disease.

The present indications are that the general yellowing of leaves and the unhealthy appearance of diseased palms is caused by the interruption to the conduction of water and nutrients to the leaves of such palms, because their xylem vessels are plugged with tyloses. This is supported by the fact that Nethsinghe (1966) has found that coconut Leaf Scorch and the general yellowing of leaves, which sometimes precedes the disease, is not primarily related to any nutritional disorder, the most common cause of leaf discolouration.

Leaf Lesions

A number of fungi were isolated from the necrotic lesions on leaves. The fungi most frequently isolated were different species of *Aspergillus*, *Botryodiplodia*, *Fusarium*, *Pestalozzia* and *Penicillium*. The usually harmless *Echidnodes Cocces* was always present on necrotic areas, but was not considered in these studies. Each of the other frequently occurring fungi were obtained in pure culture and leaf inoculations carried out with them. Two types of inoculations were made, the first on intact leaves and the second on leaves injured with a sterilized pin. These experiments were repeated six times with each fungus, but no infections were recorded.

These results indicate that the fungi isolated from the lesions are either saprophytes or essentially secondary parasites, which attack palms weakened by other causes. The fact that all the fungi isolate from necrotic areas are weak parasites eg. *Botryodiplodia theobromae* and *Pestalozzia* spp. or essentially saprophytes eg. *Aspergillus* spp. and *Penicillium* spp., supports this surmise. Further studies may be carried out on this subject to confirm this conclusion as it is essential at this stage to eliminate the possibility that the disorder is entirely a leaf disease, caused by a fungal or bacterial pathogen.

DISCUSSION AND CONCLUSIONS

Symptoms similar to Leaf Scorch Decline can be caused by nutritional imbalance, infection by a virus, a leaf or root disease pathogen (bacterium, fungus or nematode), a physiological disorder or a pest infestation. Each of these alternatives are to be carefully investigated in order to establish the cause of the disease.

The results of Nethsinghe's (1966) studies to date indicate that leaf Scorch Decline is not a deficiency disease. The studies described here, on the anatomy of the root and stem of healthy and diseased palms have supported the above conclusions ; as they have shown that leaf yellowing can be caused by starvation of the crown, through the interruption of the sap stream, as a result of tylose formation. The leaf symptoms on Fomes-infected rubber trees are similar to the yellowing of coconut leaflets, which is sometimes associated with this disease. This shows that it is important to study the root disease aspect of this disease in great detail, in spite of the negative results obtained to date from root inoculation experiments with fungi, isolated from the roots and soil around diseased palms.

The symptoms of Leaf Scorch Decline, in some aspects, resemble those produced by virus infection on certain plants. Therefore, it is important to study the possibility of this being a virus disease. In this connection (1) the expressed sap of healthy and diseased plants should be examined under the electron microscope for the presence of virus particles in the latter. This should be accompanied by (2) cross inoculation trials for the transfer of the virus, if any, to alternative hosts ; (3) serological studies on the sap of diseased and healthy palms, similar to those carried out by Peries and Brohier (1965) on *Hevea* viruses, and (4) the inoculation of healthy coconut seedlings with the sap of diseased palms, in order to establish whether the disease is caused by a virus.

The information available at the commencement of this study indicated that coconut Leaf Scorch Decline was not a disease caused by a foliar pathogen (Kirthisinghe 1966). The present studies have confirmed that the likelihood of the leaf symptoms being produced by a foliar pathogen is remote, as no primary pathogen has ever been isolated from diseased leaves. Therefore it is more likely that the necrotic lesions on the leaves of affected trees are secondary symptoms. The necrotic areas may be infected by various weak parasites and saprophytes, but the primary cause of the disease appears to be quite distinct.

Robertson (1965) has ruled out the possibility that the disease is caused by a nematode infection. The studies described here have supported his views ; as no nematode was found to be constantly associated with the diseased palms examined.

It therefore appears that this is either a physiological disease or one caused by a pest or a root parasite. It is clear that any comprehensive study of this problem will be incomplete without investigating its physiological aspects and an analysis of the pests associated with it. However, the results of this investigation have indicated that Leaf Scorch Decline is likely to be a root disease. The abnormal distribution of soil fungi, with a predominance of Fusarium spp., round diseased palms, the root tip necrosis, also largely associated with Fusarium spp., the plugging of vessels with tyloses in diseased palms, the typical starvation sym'ptoms on the fronds of affected palms, and the slow development of the symptoms of the disease, all support this conclusion. The consistent association of *Fusarium* spp. with this disease, both in the soil and in necrotic roots, strongly indicates that it is caused by one or more species of this fungus.

Fusarium spp. have been isolated from healthy rootlets too, but this is normal for an ubiquitous soil inhabiting fungus. However in all cases investigated, advanced symptoms of Leaf Scorch Decline, such as failure to produce nuts and collapse of the crown, were always recorded on trees showing advanced root decay, and *fusarium* spp. have been isolated from a majority of such decaying roots. It has also been observed that Leaf Scorch is frequently associated with abnormal soil conditions (Kranz 1967) and, as stated above, symptoms of the disease take an unusually long time to develop. These facts support the conclusion that the disease is caused by a week root parasite such as a Fusarium spp. as it is well established that *fusaria* usually infect plants lacking in vigour as a consequence of a high water table or a lack of nutrition. The symptoms of Leaf Scorch Decline have been observed only on trees above 20 years of age, and all attempts at transferring the disease artificially to healthy trees have failed. Studies on the spread of the disease have given inconclusive results, and the indications are that it spreads very slowly, if at all, from tree to tree (Kirthisinghe 1966). This suggests that the causal agent of the disease needs to be closely associated with the host over a long period before disease symptoms appear on the latter. It is therefore possible that the disease is caused by a weak parasite or even a saprophyte, such as a Fusarium spp., under special circumstances. The coconut palm is a perennial, which occupies the same area of soil for a number of years. It is possible that the soil conditions could change over the years under such circumstances giving rise to an environment which enables a harmless saprophyte to become a weak parasite, which affects the root system. This condition can be aggravated over a number of years, until the original saprophyte or weak parasite assumes the role of a pathogen. In such a case it would not be possible to fulfil Koch's Postulates regarding a plant pathogen. However, Garrett (1959) has referred to " the despotism of Koch's Postulates—a despotism which began beneficially by insuring the essential observances of strict rules of evidence in the conduct of inoculation experiments, and ended by restricting enterprise ". Therefore, the failure to transfer the disease to healthy plants artificially should not act as a deterrent to further studies on the possibility of this being a root disease caused by one or more species of Fusarium, which are generally saprophytes or weak parasites.

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