KFRI Research Report 25

STUDIES ON THE LITTLE LEAF DISEASE OF EUCALYPTS

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PEECHI, THRISSUR

March 1985 Pages:15

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ABSTRACT

A little leaf disease was recorded in plantations of *Eucalyptus tereticornis* and *E. grandis* at many localities in Kerala. The possibility of the association of mycoplasma like organisms (MLOs) with this disease was investigated in detail. General anatomical studies indicated necrosis and excessive formation of phloem in diseased plants. Diseased phloem tissues showed positive reaction to Dienes' stain in the form of dark blue spots while fluorochrome, aniline blue gave bright fluorescent spots. However, Hoechst 33258, a DNA binding fluorochrome gave negative reaction. Disease could not be transmitted either through grafting or dodder. Tetracycline infusion in affected trees gave temporary remission of the disease symptoms. In transmission electron microscopy MLOs were found in the phloem of the diseased tissue. The present study has confirmed the association of MLOs with the litttle leaf disease of eucalypts.

INTRODUCTION

In recent years yellows type of diseases in plants have gained importance due to involvement of mycoplasmas or allied pathogens as their causative agent (Whitcomb and Davis, 1970; Maramorosch *et al*., 1970; Davis and Whitcomb, 1971; Ghosh and Raychaudhuri, 1972; Hampton, 1972). In India, more than **30** plant species are reported to be affected by yellows pathogens; some of these diseases are economically important (Ghosh and Raychaudhuri, 1974). Mycoplasmal etiology of yellows infected plant diseases was first suspected by a group of Japanese workers headed by Prof. H.Asuyama. Ultrastructural morphology of the organisms in the diseased tissue and remission of the symptoms by tetracycline therapy are the two evidences for the mycoplasmal etiology of these diseases (Doi *et al*., 1967; Ishiie *et al.,* 1967). Electron microscopic studies by the Japanese workers showed the association of mycoplasma like organisms (MLOs) in some of the yellows disease infected plants

Yellows pathogens, which thrive in the phloem, induce a wide range of visible symptoms characterised by disturbances in translocation of food material as well as growth hormone. Some of the symptom expressions of the yellows infected trees are: suppression of apical dominance and sprouting of axillary buds forming excessive branching, littling of leaves, shortening of internodes, induction of sterility and general yellowing of the foliage. The sequence of symptom expression and progress of the disease in sandal trees have been discussed elsewhere (Ghosh *et al.,* 1981). In nature the disease is transmitted by phloem feeding insect vectors (Maramorosch *et al.,* 1970; Whitcomb and Davis, 1970; Ghosh *et al*., 1984). Under laboratory conditions the disease can be transmitted by grafting or through the agency of angiospermic parasite, dodder (Ghosh *et al.,* 1977; Dijkstra and Lee, 1972).

Importance of eucalypts in Kerala

Eucalypt (family: Myrtaceae) is one of the fast growing species introduced in India from Australia. At present eucalypts are cultivated in almost all the states in India. *Eucalyptus tereticornis, E. globulus, E. grandis* and *E. camaldulensis* are the important species tried in India. *E. grandis* was first introduced in Kerala State in large scale in 1948 (FAO, 1979). So far about 14,800 ha of *E. grandis* and 23,800 ha of *E. tereticornis* have been raised in plantations (Karunakaran, 1982).

The most serious diseases of eucalypts in Kerala are pink disease caused by the fungus *Corticium salmonicolor* and leaf and shoot blights by *Cylindrocladium* spp- (Bakshi, 1976; Sharma *et al.,* 1984). Though several virus diseases have been reported from *Euculyptus* spp. (Fawcett, 1940; Foddai and Marras, 1963). they are not as devastating as the fungal diseases. In India three virus diseases of *E. citriodora,* were described by Sastry *et al*.(1971). These include mosaic, leaf crinkle, and little leaf disease. The little leaf disease reported on *E. citriodora*, from Karnataka was graft transmissible. The diseased plants are stunted with excessive branching and with characteristic iittling of leaves.

The little leaf disease of *Eucalyptus* is occasionally found in eucalypts in Haryana, Rajasthan, Uttar Pradesh, Tamil Nadu, Karnataka and Kerala. In various parts of Kerala, the disease has been noticed on several species of eucalypts. The infected trees remain stunted and sterile and are characterised by extreme reduction of leaves and excessive sprouting of axillary buds (Fig. 1). This disease was observed in *E. tereticornis* plants in Arissery 1978 plantation in Wadakkancherry range of Trichur Forest Division and in a mixed plantation of *E. grandis* and *E. eugenoides* in Thalayar Tea Estate, in Munnar Forest Division. Eventhough this disease is not devastating at present, it could become a serious threat due to monoculture and uncontrolled growth of vector population

Before studying the management aspects of this disease in greater detail, an in-depth study was taken up to find out the exact nature of causal organism. The present study was focussed mainly on four aspects, viz., (1) detection of the disease by light and fluorescence microscopy, (2) ultrastructural studies, (3) transmission through grafting and dodder and (4) tetracycline therapy.

MATERIALS AND METHODS

Diseased trees (naturally infected) of ***E. grandis*** occurring in the plantation (attitude 1500 m above msl) of Munnar Forest Division were used as source of samples of diseased ***E. grandis*** plant for various experiments. A few diseased ***E. tereticornis*** plants from Wadakkancherry range (Arissery, 1978 plantation) of Trichur Forest Division (altitude 100 m above msl) were uprooted, brought to the institute and established in the nursery for disease transmission and histopathological studies.

1. Detection of diseased tissues

(i) Histopathological studies

Samples from young internodal region about 2 to 3.0 cm from the apex were taken from diseased and healthy *E. grandis* and *E. tereticornis* for histopathological studies.

(a) *Anatomical Studies:* Young stem bits of diseased *E. tereticornis* were fixed in formalin – acetic acid – alcohol (FAA), dehydrated in n-butyl alcohol series and embedded in paraffin wax Sections (8 to 10 μm) were cut using rotary microtome, stained with modified crystal violet - erythrocin stain (Jackson, 1926) and mounted in DPX mountant and viewed under Leitz Dialux microscope. Phloem tissues were examined for any deformities.

For studying the quantitative changes in phloem, sections were viewed under projection microscope; the image of sections were drawn on a graph paper and the percentage of area occupied by phloem out of the total area assessed

(b) *Dienes'staining reaction:* Dienes'stain normally used for detection of animal mycoplasmas (Dienes *et al.,* 1948) contained 2.5 g of methylene blue, 1.25 of Azure II, 10 g of maltose, and 0.25 g sodium carbonate in 100 ml of distilled water. Hand cut sections of fresh tissues of diseased and healthy *E. **grandis*** were directly stained in 0.20% solution of filtered Dienes' stain for 10 minutes, sections were then washed and mounted in distilled water and viewed under microscope.

(i) Fluorescence microscopy

Aniline blue, a fluorochrome used for detecting callose in the phloem of mycoplasma infected plant tissues (Hiruki and Shukla, 1973) was used in our studies. Free handcut sections of fresh tissues were immediately heat–killed in boiling water and stained in 0.01% aniline blue in 1/15 M phosphate buffer (Ghosh *et al.,* 1974 & 1984). Stained sections were viewed and photographed under Leitz Dialux fluorescence microscope.

Hoechst 33258. a substitute to DAPl (4-6-diamidino-2' phenylindole), a DNA binding fluorochrome reported to detect mycoplasmal DNA (Russel ***et al.,*** 1975: Hiruki, 1982) was also used in our studies. Free handcut sections were fixed in

3.0% gluteraldehyde in cacodylate buffer at 4°C for 4-2 hours. Sections were then washed in 0.1 M phosphate buffer and stained in Hoechst 33258 prepared in 0.1 M phosphate buffer containing 1 to 100 μg/ml for 15 - 20 minutes and viewed under Leitz Dialux fluorescence microscope.

II. Ultrastructural studies

Two millimeter bits of young stem and petiole of diseased and healthy *E. grandis* trees were fixed in cold 2.50%gluteraldehyde in cacodylate buffer (pH 7.0) for 2 hours. Then they were washed thoroughly and postfixed in 1% osmium tetroxide for 1 hour, After washing, the materials were dehydrated in acetone series and embedded in TABB embedding resin (Ghosh *et al.,* 1977).

Ultrathin sections were cut with LKB III Microtome, differentiated with urany acetate and lead citrate and the sections were viewed under Philips EM 300 and photographed.

III. Transmission Studies

(i) Graft transmission

Grafting experiments were carried out on 6-month-old Seedlings of *E. grandis* and *E, tereticornis.* Side grafting and wedge grafting methods were used. All the grafted seedlings were kept under shade for establishment.

(a) *Side grafting:* Diseased twigs (scion) similar in size of root stock (healthy seedlings) were cut to 'v' shape. Slant cut of appropriate length was made on the stock. The scion was inserted into the slit and carefully tied with polythene strip. Fifty grafts were made and scion of 25 grafts were covered with polythene bags. The grafting was done during various months of the year stretching from warm, rainy to cool weather conditions.

(b) *Wedge grafting:* The tip of scion was scrapped into 'v' shape. The terminal portions of the stem of the stock Seedlings were cut at 1/3 of the height from top and a vertical slit was made. The scion was inserted into the slit carefully and tied with polythene strip. Fifty grafts were made and scion of 25 grafts were covered with polythene bags. The experiment was repeated as done in the case of side grafting. The diseased twigs were also grafted to *Vinca rosea, Mirabilisjalapa* and *Santalum album.*

(ii) Dodder transmission

Cuscuta chinensis and *C. reflexa* were used for dodder transmission studies. *Cuscuta* seeds were treated with concentrated sulphuric acid for 10 minutes, washed thoroughly in water and kept on moist filter paper in petridishes for germination. Two to three days after germination, *Cuscuta* plants were carefully trailed on diseased twigs for establishment. Later, strands of established *Cuscuta* were trailed to 6-month-old healthy plants connecting the diseased plants to the healthy ones.

The plants were kept till the dodder withered away. Later, the plants were shifted to large glass cages for symptom development.

Cuscuta strands trailed on diseased *E. grandis* and *E. tereticornis* were also trailed on healthy *E. tereticornis* and *E. grandis* respectively and also on healthy seedlings of *Vinca rosea*, *Santalum album* and *Ziziphus oenoplea*.

All the grafted seedlings and the *Cuscuta* trailed seedlings were partially pruned after 40 and 80 days for appearance of disease.

(iii) Rooting of stem cuttings

Attempts were also made to root the little leaf diseased *E. grandis* stem cuttings by treatment with indolebutyric acid (IBA) (Loba-Chemie Industrial Co., Bombay) arid Seradix (B) May & Baker, Bombay). About 15 cm length of ten cuttings each, were kept dipped in 20, 50 and 100 ppm aqueous solutions of IBA for 24 hours. Likewise, 10 cuttings each were dipped in 200 and 250 ppm solution of Seradix B for 24 hours. The cuttings were planted in trays containing garden soil. The trays were kept under shade with high humidity.

IV Tetracycline therapy

Tetracycline antibiotics were infused into four diseased *E. grandis* trees in Thalayar plantation, using the tree injection device, developed in the institute (Ghosh and Balasundaran, 1980). Tetracycline HCI (IDPL, Rishikesh) @ 1g/tree and Oxytetracycline HCI Tree Injection Formula (Pfizer Ltd., Bombay) @ 3 g/tree. each dissolved in 500 ml water were infused into two diseased trees each.

RESULTS AND DISCUSSION

I. Seasonal expression of disease symptoms

Disease symptoms in *E. tereticornis* were found to be influenced by climatic conditions. During dry summer season new sproutingson the diseased plants were less. However, some sprouts which appeared during summer had normal size leaves. At the same time sprouts during the monsoon season were invariably with typical little leaf disease symptom. The diseased plants in the Thalayar plantation *(E grandis)* survived well during the experimental period without much seasonal effect on the symptom expression.

II Detection of diseased tissues

(i) Histopathological studies

fa) ***Anatomical studies:*** Phloem necrosis was seen in diseased stem tissue (Fig. 2). Quantity of phloem in healthy and diseased stem tissues was found to be 15% and 21 % respectively. Thus there is a general increase of about 40% phloem in the diseased stem tissue over healthy stem tissue (Fig. 2) in *E tereticornis*.

The marked increase in quantity of phloem over healthy may be attributed to the fact that since the organism isa phloem pathogen, especially sieve tubes, deposition of callose may occur in damaged sieve cells. Because of the disruption in the normal phloem tissue, there might be a tendency to circumvent the damage by producing excessive phloem tissue in the diseased plants

Phloem necrosis and formation of excessive phloem in yellows infected trees have been noted earlier by Schneider (1973 & 1977) and Dijkstra and van der Want (1970). Ghosh *et al* (1974) studied the anatomical changes occurring in witches' broom disease affected *Mirabilis jalapa* and reported marked degeneration of phloem tissue and pronounced secondary growth at the same time.

(b) *Dienes' staining reaction:* Dienes' staining reaction gave excellent differentiation of the diseased tissue in our study. Sections of phloem from infected tissues of *E. grandis* when reacted with Dienes' stain showed several regularly distributed distinct blue stained areas (Fig. **3).** No such distinct area was seen with healthy tissues. When magnified, dark stained individual cells in the phloem were clearly seen.

Positive Dienes' reaction was also obtained by Deeley *et al.* (1979), when working with yellows disease, as compared to bacterial and virus diseases. Raju *et al.* (198l 1working with brittle root of horse radish could prove the association of spiroplasma with the disease using Dienes' stain. In the present study the phloem of plants containing the Dathogen stained deep blue whereas none of the diseases due to virus, bacteria and fungi, exhibited phloem staining.

During our study we have checked a viral disease namely silver mosaic and also other littling symptoms in eucalypts by Dienes' reaction and found that the results were negative. Obviously, mycoplasmaf association is not involved in these diseases.

(ii) Fluorescence microscopy

Diseased and healthy sections when stained with aniline blue gave excellent differentiation under fluorescence microscope. Bright yellow green fluorescent spots were observed both in outer and inner phloem of diseased tissue of *E. grandis* and *E. tereticornis* whereas there were only very little fluorescent spots in outer and inner phloem of healthy tissues (Fig. 4 & 5).

Aniline blue, reported as a callose-specific fluorochrome (Eschrich and Currier, 1964) was used by Hiruki and Shukla (1973) to differentiate between phloem from healthy and diseased bleeding heart *(Dicentra spectabilis)* by showing more fluorescence in the diseased sieve elements than the healthy. Hiruki and Dijkstra (1913) and Hiruki *et al.,* (1974) obtained similar response when sections from *Vinca* plants infected with the sandal spike MLOs were stained with aniline blue. The presence of MLOs in the phloem cells was confirmed by examining ultrathin sections under electron microscope. Accumulation of callose, known chemically as β-1, 3- D glucan (Aspinall and Kessler, 1957; Feingold *et al* , 1958) may also happen due to virus infection (Hiruki and Tu, 1972) and fungi (Aist and Williams, 1971). Ghosh *et al.,* (1974) observed best differentiation in sections stained with aniline blue showing br0ight yellow green fluorescent spots in diseased phloem of witches' broom affected *Mirabilis jalapa.* Dijkstra and Hiruki (1974) showed a marked difference in fluorescence in phloem of healthy and sandal spike diseased trees and discussed about its diagnostic values for early detection of disease.

Hoechst 33258 did not give any positive DNA fluorescence. In both the species we could not see any DNA specific fluorescence. Russell *et al.* (1975) showed that DAPI (4–6 diamidino-2-phenylindole), a DNA binding fluorochrome could be used to detect mycoplasmal contamination of tissue cultures. Seemuller (1976) was the first to recognise that DAPI and a benzimidole derivative, Hoechst 33258, could be used as fluorcchromes to detect MLOs in phloem of trees affected by pear decline disease. hiruki (1982) used aniline blue and DAPI as fluorochromes for detectina MLOs in mulberry dwarf and paulowniawitches' broom. He found that paulownia witches' broom gave positive fluorescence for aniline blue but not for DAPI while mulberry dwarf gave positive fluorescence with DAPI but not with aniline blue. In our study also aniline blue fluorochrome worked very well and excellent disease specific results were obtained, whereas with Hoechst 33258 the extent of fluorescence was rather obscure and not reliable for disease diagnosis. The cause of this possible discrepency needs further investigation.

From the observations, it is possible to say that the methods like general anatomical studies, Dienes staining reaction and fluorescence microscopy studies

can be used as routine diagnostic tests for little leaf diseases caused by mollicutes. Electron microscopy, used to demonstrate the presence of MLOs in phloem of diseased tissue and tetracycline therapy used to find out remission of the disease symptoms, eventhough are the most reliable methods for confirming the etiology of little leaf diseases, they are time consuming and expensive. In such cases, anatomical results showing phloem necrosis and excessive formation of phloem, Dienes' positive reaction and aniline blue positive fluorescence can very well be used to identify little leaf diseases of eucalypts. During our study we have come across cases of littiing of eucalypt leaves from places like Mavinhalla, Munnar, etc and other virus diseases resembling little leaf symptoms. In all cases, using the simple anatomical methods we could successfully differentiate little leaf disease induced by mollicutes from other little leaf diseases.

III. Ultrastructure studies

Thin sections of phloem tissue of naturally infected little leaf diseased eucalypts revealed MLOs in sieve tubes. No MLO was found in comparable healthy eucalypt material. The abundance of MLO varied from cell to cell. The concentration of MLO in cells was less. In size and morphology, these bodies are similar to mycoplasma like organisms (MLOs) reported to occur in phloem cells of plants affected by yellows type of diseases (Fig 6). No bacteria, virus-like particles or fungi were detected from any of the sections examined.

Recently, Maramorosch *et al,* (1982) reported a little leaf disease of *E. tereticornis* from Haryana, Rajasthan and Uttar Pradesh associated with mycoplasmas. Electron microscopy of thin sections from diseased leaves revealed the presence of typical MLOs in sieve elements.

IV. Transmission studies

(i) ***Graft transmission:*** None of the scion (diseased twigs) grafted on healthy eucalypts seedlings established The scions remained green for a week and slowly wilted and dried. In all the grafted seedlings including those of *V. rosea* and *S. album* no symptom was observed indicating that disease could not be transmitted through dose contact with diseased phloem tissue. Graft transmission of little leaf disease of *E. citriodora* was reported by Sastri *et al.* (1978). McCoy *et al.* (1973) failed to transmit the pigeon pea witches' broom by grafting.

(ii) *Dodder* ***transmission:*** After an initial establishment for 2 weeks on diseased as well as on healthy twigs, the *Cuscuta* started drying up. Though *Cuscuta* survived for about 10 days on the healthy seedlings after its initial establishment on diseased plants, disease transmission did not occur even after pruning and keeping under observation for 6 months. It seems that *eucalypts* are not good host for the species of *Cuscuta* used. Dijkstra and Leo (1972) transmitted sandal spike disease to V. *rosea* and back to sandal through dodder and the MLO was detected in the diseased plant tissue and dodder. Ghosh *et al.* (1977) found multiplication of citrus greening organism in phloem cells of dodder.

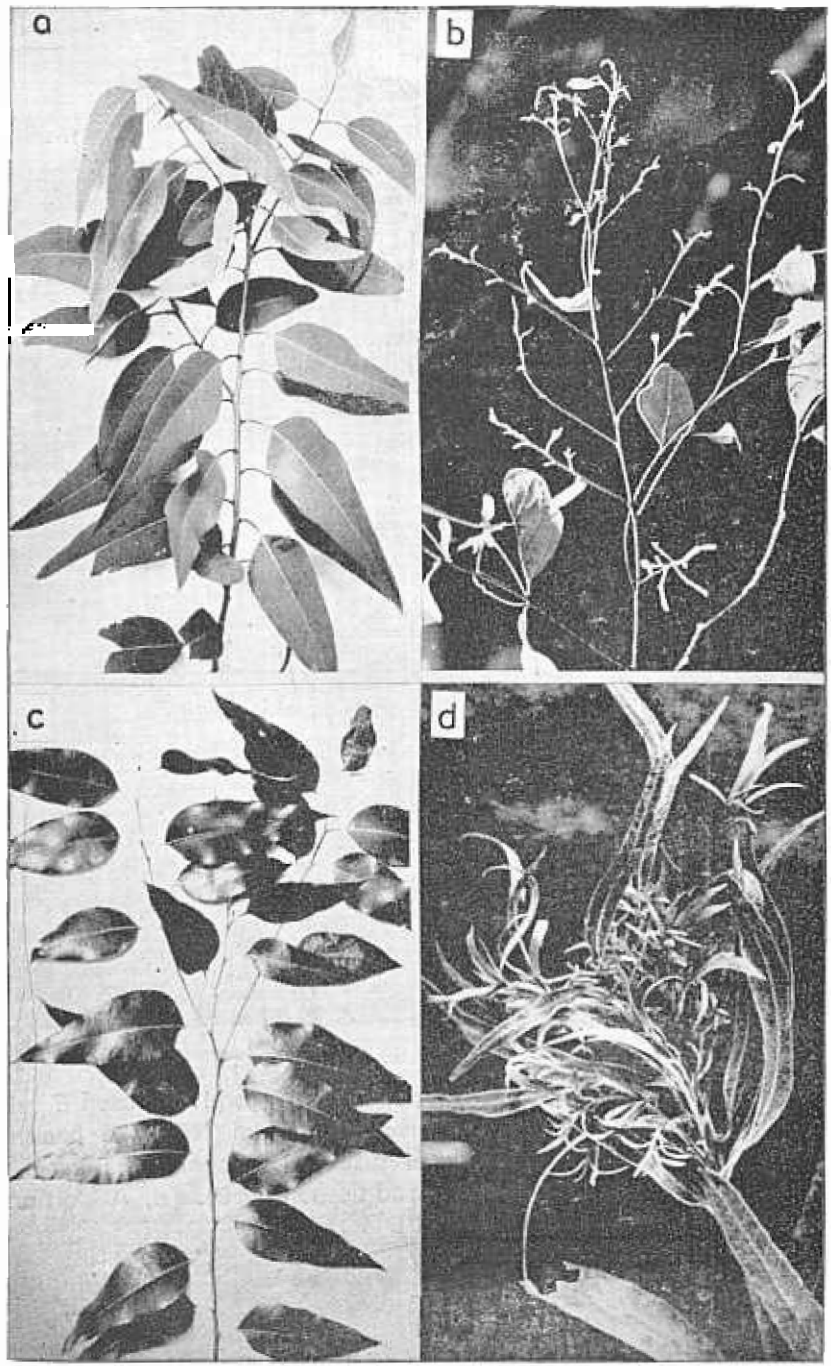

Fig 1. Typical symptom of little leaf disease. **a and b,** Healthy and diseased *E. tereticornis* shoots. **c and d,** Healthy and diseased *E. grandis* shoots.

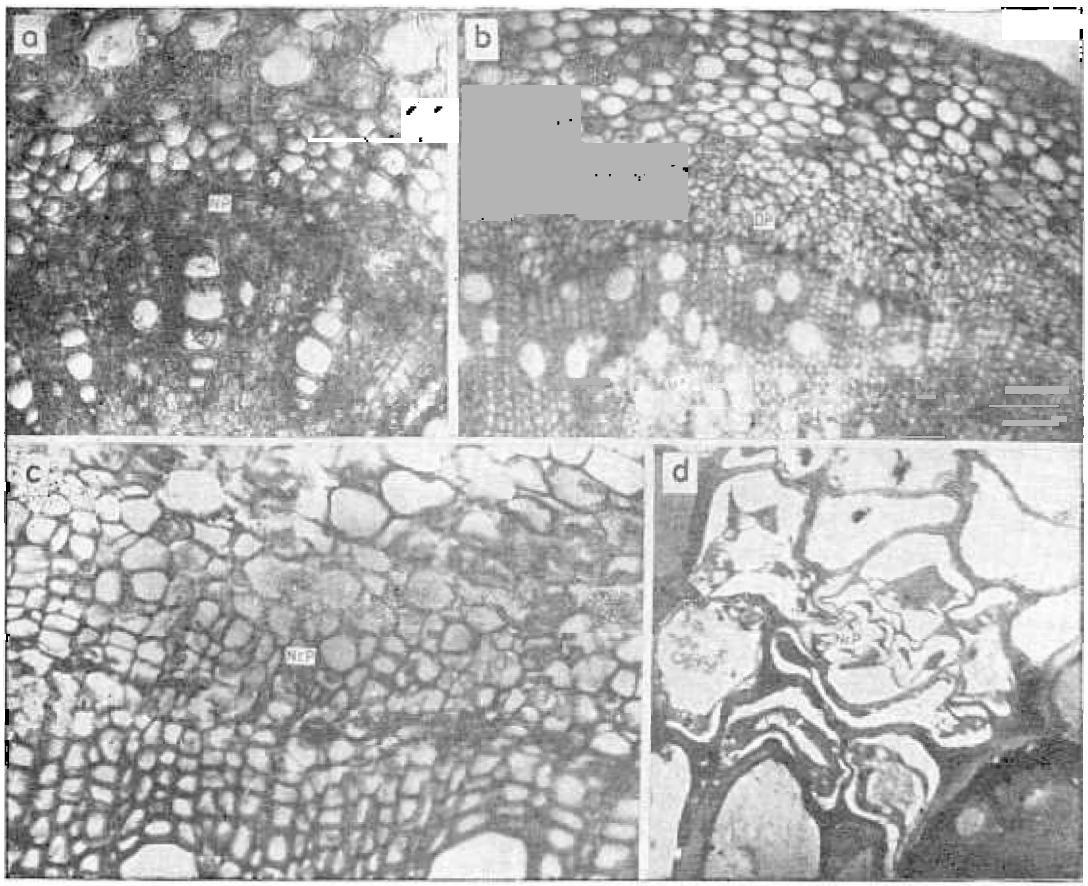

Fig. 2. Photomicrographs of cross sections of healthy and diseased *E. tereticornis* stem showing phloem tissue. **a**, Normal phloem (NP) of a healthy tissue (X150). **b**, Increased quantity of diseased phloem (DP) tissue (X150.) **c**, Necrotic phloem (NcP) of diseased tissue (X350). **d**, A portion of the necrotic phloem magnified (X2200).

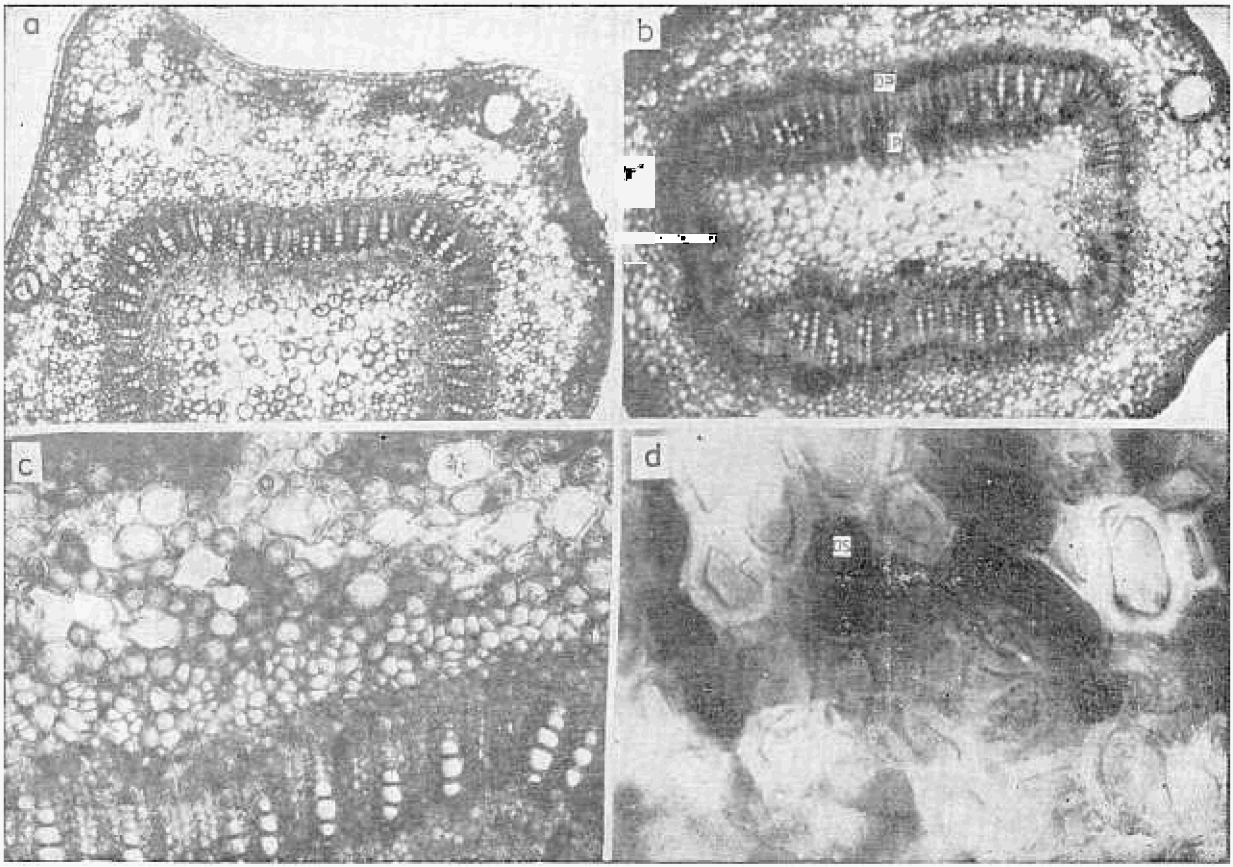

Fig. 3. Photomicrographs of cross sections of diseased and healthy *E. grandis* stem, showing Dienes' reaction in phloem tissues. a, Healthy plant tissue (X60). b, Diseased plant tissue (X60); note the dark spots (DS) present in the outer and inner phloem (OP, IP) tissues. c, A portion of the healthy tissue magnified (X150). d, A portion of the diseased phloem tissue magnified (X960); note the dark spots (DS) present in the phloem elements.

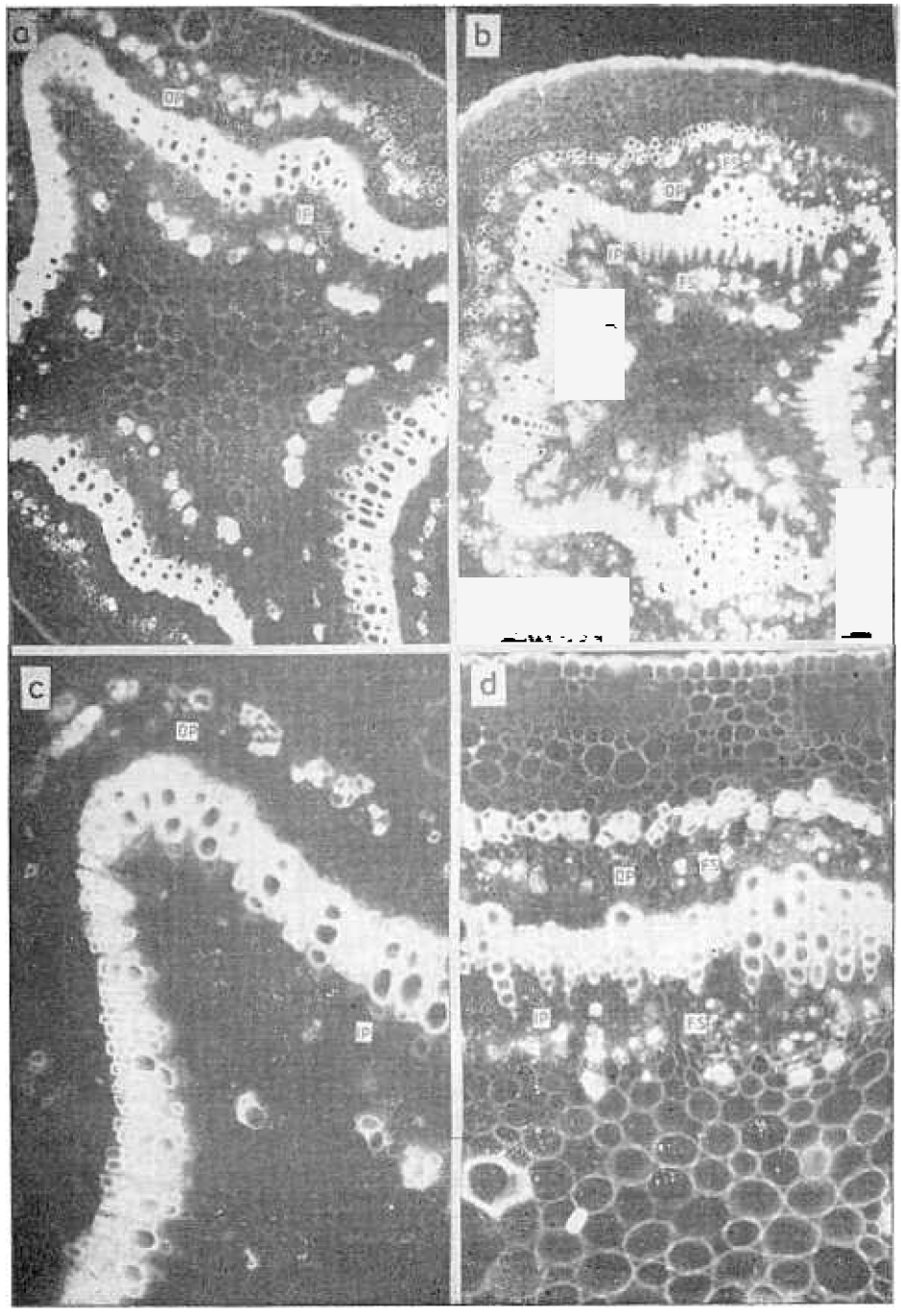

Fig. 4. Fluorescent micrograph of cross sections of *E. grandis* stem. **a**, Healthy tissue (X50). **b**, Diseased tissue (X50). **c**, A magnified portion of healthy tissue (X130); the phloem region shows only very few fluorescent spots. **d**, A magnified portion of diseased tissue (X130); note numerous bright fluorescent spots (FS) in the phloem.

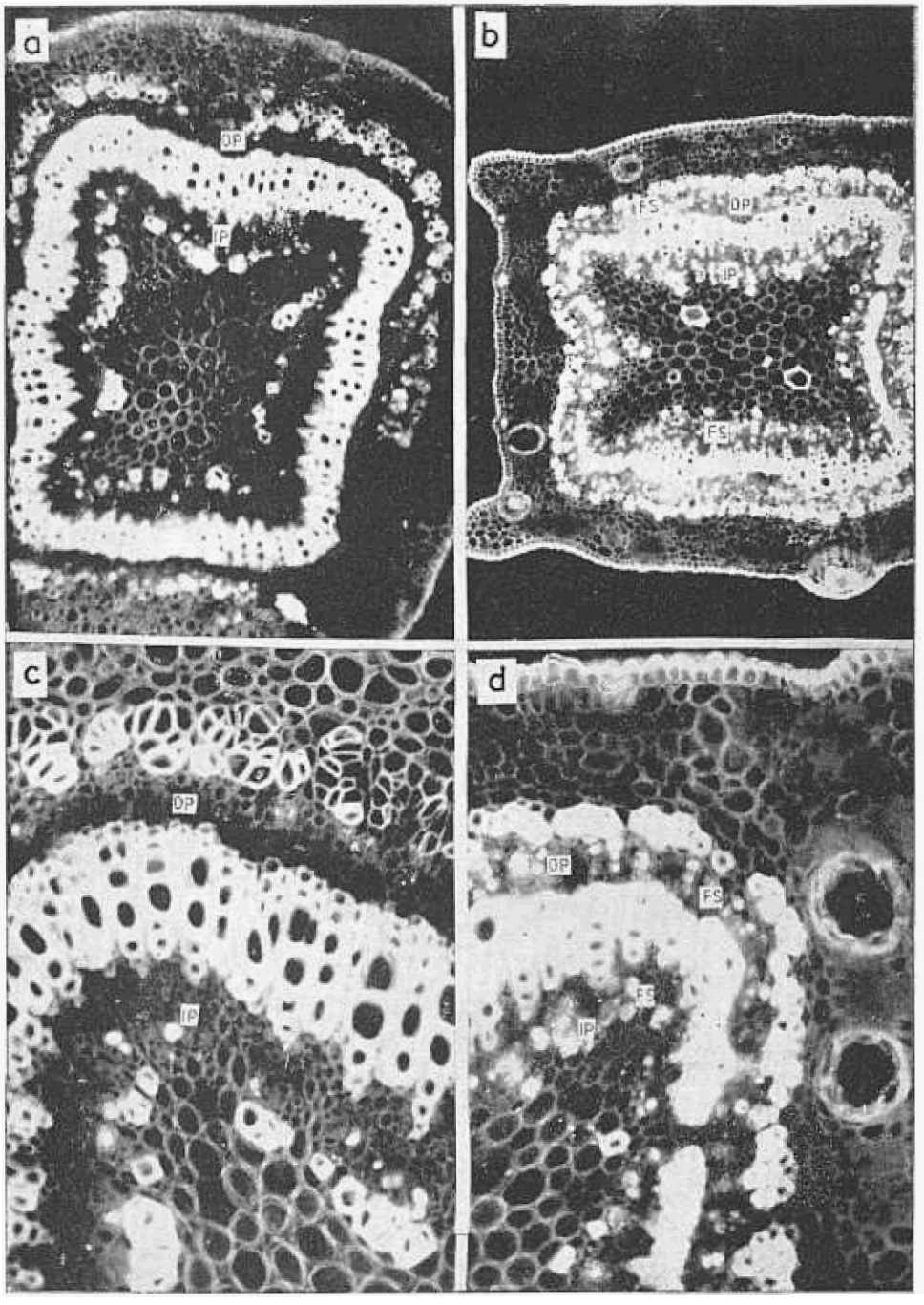

Fig. 5. Fluorescent micrographs of cross sections of *E. tereticornis* stem. **a**, Healthy tissue (X50). **c**, A magnified portion of a healthy tissue (X130); the phloem regions show only very few fluorescent spots. **b**, Diseased tissue (X50). **d**, A magnified portion of diseased tissue (X130) note the numerous bright fluorescent spots (FS) in the phloem.

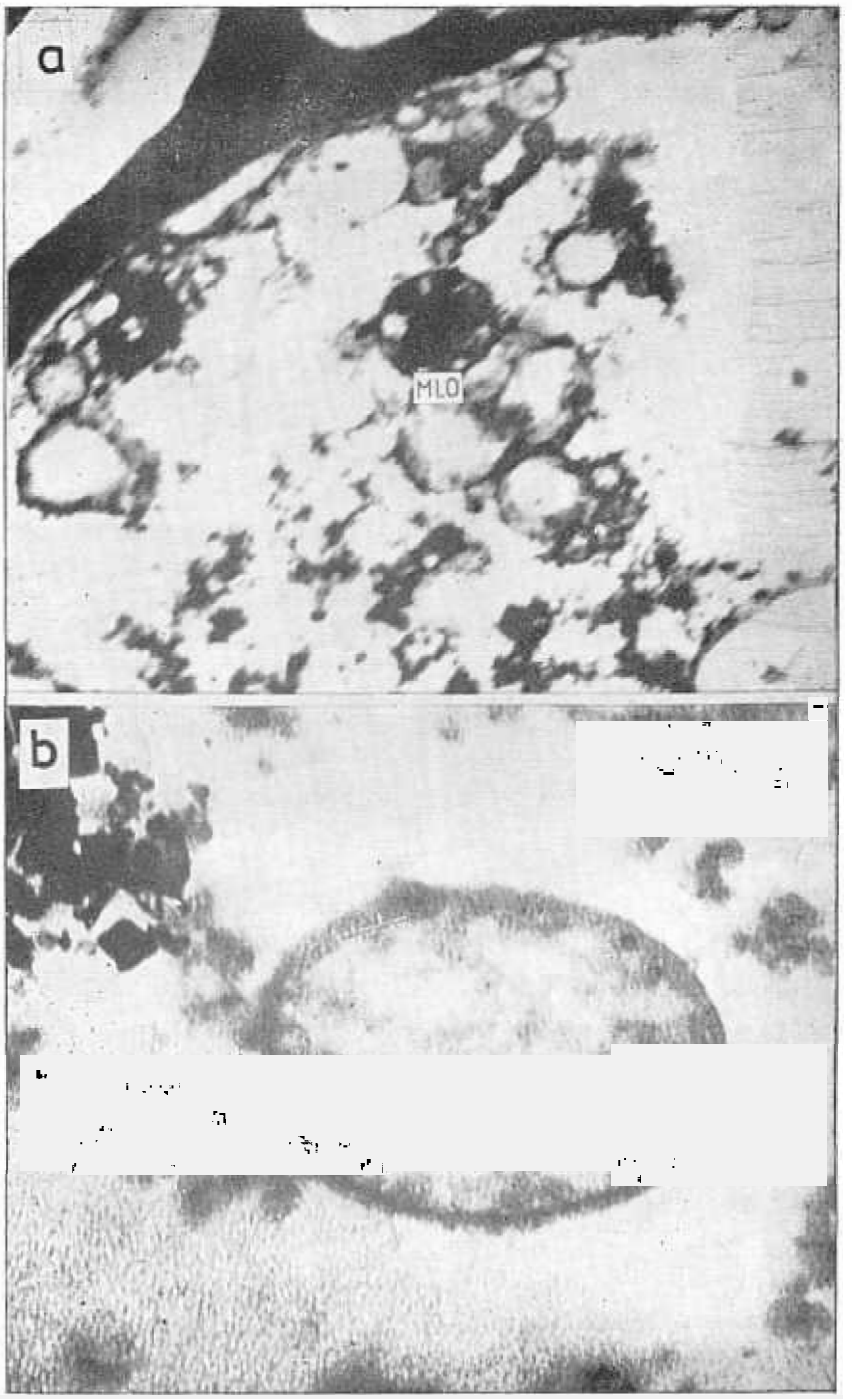

Fig. 6. Electron micrograph of diseased *E. grandis* phloem tissue. a, Mycoplasma like organisms (MLOs) in sieve cells (X11,000). b, One MLO magnified (X100,000).

(iii) *Rooting of stem cuttings:* Attempts to root cuttings from the diseased and healthy plants also failed. McCoy ***et al.*** (1983) could not root stem cuttings from witches' broom affected Pea plants.

V Tetracycline therapy

Eventhough the presence of MLOs in diseased leaves of *E. tereticornis* was shown by Maramorosch *et al.* (1982), no tetracycline therapy was undertaken to show the temporary remission associated with plant MLO diseases.

In the present study, we found that trees treated with Tetracycline HCl at 1.0 g/500 ml and oxytetracycline HCl (OTC) tree injection formuia 3 g/500 ml showed new healthy growth 30 days after treatment. The remission lasted for 60-90 days, after which the symptoms reappeared.

In the absence of a viable culture to prove the Koch's postulate, the etiology of yellows disease could be proved by electron microscopy and tetracycline therapy. A number of plant diseases have been attributed to be caused by mycoplasma like organisms by electron microscopy and tetracycline therapy (Doi et al., 1967; Ishiie *et al.*, 1967; Davis *et al.,* 4968). In india remission of symptoms of sandal spike using tetracycline antibiotics was obtained by Raychaudhuri *et al.* (1972) and Rao *et al.* (1972).

In Florida, McCoy (1972) found the remission of lethal yellowing in coconut palm treated with tetracycline antibiotics. La *et al.* (1976) obtained control of witches' broom of jujuba with OTC injection. Yi, *et al.* (1981) discussed the therapeutic effects on witches' broom disease of paulownia with OTC injection on stern of infected tree. Rosenberger and Jones (1977) also found the symptom remission in 'X' diseased peach trees. Chemical treatment for the control of plant mycoplasma diseases has been recently reviewed by Sinha (1979), Nair (1981) and McCoy and Williams (1982).

VI. Natural recovery of little leaf disease

Littling of eucalypt leaves can be due to various reasons. In eucalypt nurseries, little leaf affected plants are quite common in Kerala (Sharma *et al.*, 1983). In such cases, the trees either get recovered naturally or die in the seedling stage. From Zambia, a little leaf condition of young eucalypts is reported which causes, gross stunting of 1-2 year old plants in the field, reducing them to balls of stunted reddened leaves. These plants usually recover naturally within a year or two, (I. A. S. Gibson, *Personal communication)* Apparent natural recovery or spontaneous remission of little leaf disease of *E tereticornis* was noted in the field specially during dry summer. In such plants the disease symptoms reappear in the favourable seasons. In summer MLOs may degenerate thereby trees recover and at favourable seasons MLOs rejuvenate again, and the plants develop the symptoms afresh. Schaper and Seemuller (1982) observed that in plants affected by apple proliferation and pear decline, MLOs survive mainly in root system of affected plants during severe winter and that they spread from there to the aerial parts in the spring. Natural recovery of little leaf disease was not observed in *E. grandis* plantations probably because of the absence of a hot summer in Munnar, being situated at high elevation and the evenly distributed rainfall throughout the year.

SUMMARY AND CONCLUSION

Little leaf disease of eucalypts was observed in some plantations of Kerala. The materials for present study were obtained from a *E.tereticornis* plantation and a mixed plantation of *E. grandis* and *E. eugenoides*. Transmission attempts through grafting and dodder failed to transmit the disease to healthy seedlings of *E. tereticornis* and *E. grandis*. General anatomical studies showed excessive formation of phloem and phfoem necrosis in diseased tissues only. Dienes'staining reaction showed regularly distributed distinct blue areas in diseased phloem tissue. Fluorescence microscopy using aniline blue as callose binding fluorochrome 'showed bright yellow green fluorescent spots in outer and inner phloem of thin ssctrons from diseased tissues. But in healthy ones such spots were very less. Hoechst 33258, a DNA binding fluorochrome gave negative reaction. Ultrastructure studies showed pleomorphic mycopiasma like organisms (MLOs) in sieve elements of diseased tissues only. But their Concentration was very low. Tetracycline infusion into diseased trees showed temporary remission of disease symptoms. Based on the light microscopic and ultrastructural studies and remission of the disease symptoms by tetracycline, association of mycoplasma like organism (MLO) with the little leaf disease of *E. tereticornis* and *E, grandis* is confirmed. Even though. electron microscopy is the most reliable and confirmatory method to show MLOs in phloem, other simple histopathological methods like fluorescence microscopy using aniline blue, Dienes'staining reaction and general anatomical studies can be used for routine detection of little leaf diseases caused by rnollicutes or allied pathogen,

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