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ELECTRON MICROSCOPY OF LEAF SECTIONS FROM KERALA WILT DISEASED COCONUT PALMS

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ABSTRACT

Electron micrographs of thin sections of coconut palm leaves from Kerala root wilt diseased trees revealed the presence of two types of submicroscopical particles. One type was the commonly encountered phytoferritin which forms characteristic accumulations in diseased leaves. The other represented virus-like particles. Further studies might reveal whether the virus-like particles are constantly associated with the Kerala root wilt disease.

INTRODUCTION

The disease of coconut palms in the state of Kerala, south India, has been known since 1876 (Anonymous, 1976: Maramorosch, 1974). Its aetiology is still uncertain and various hypotheses have been proposed, including physiological causes, fungi, bacteria, viruses, viroids, mycoplasma-like agents, and toxins. The present note reports the attempt to visualize an extraneous disease agent by thin section electron microscopy techniques. Although this procedure has been used successfully in determining the association of mycoplasma-like agents with numerous plant diseases since 1976 (Maramorosch, 1976; Muller, et al., 1975), as well as the association of viruses with certain plant virus diseases, the results of such a study can, at best, provide an indication of the presence of morphologically distinct microorganisms or particles, but cannot prove the cause of disease. Furthermore, the absence of positive findings does not preclude the actual presence, in concentration too low to be detected by the screening techniques that could possibly be detected by other techniques. Finally, the presence and visualization of a presumptive disease agent does not necessarily mean that it is the actual causative agent, because "passenger viruses" and microorganisms introduced as chance contaminants might be observed, thus obscuring the picture. With this in mind, the results of the present investigation are presented merely as an indication of a possible association of a disease agent with the uncertain aetiology coconut wilt disease of Kerala.

MATERIALS AND METHODS

Leaf samples for electron microscopy, 1×1 mm, were obtained from freshly removed fronds of a coconut palm growing in a pocket of disease at Kalpakavady Hotel, Kalpakavady, near Alleppey, Kerala. The samples were excised from portions of leaves submerged in 1.5 per cent cacodylate buffered glutaraldehyde as described elsewhere (Hirumi and Maramorosch, 1973). The pieces were immediately placed in glass vials, filled completely with the fixative, then covered with parafilm membrane and closed with

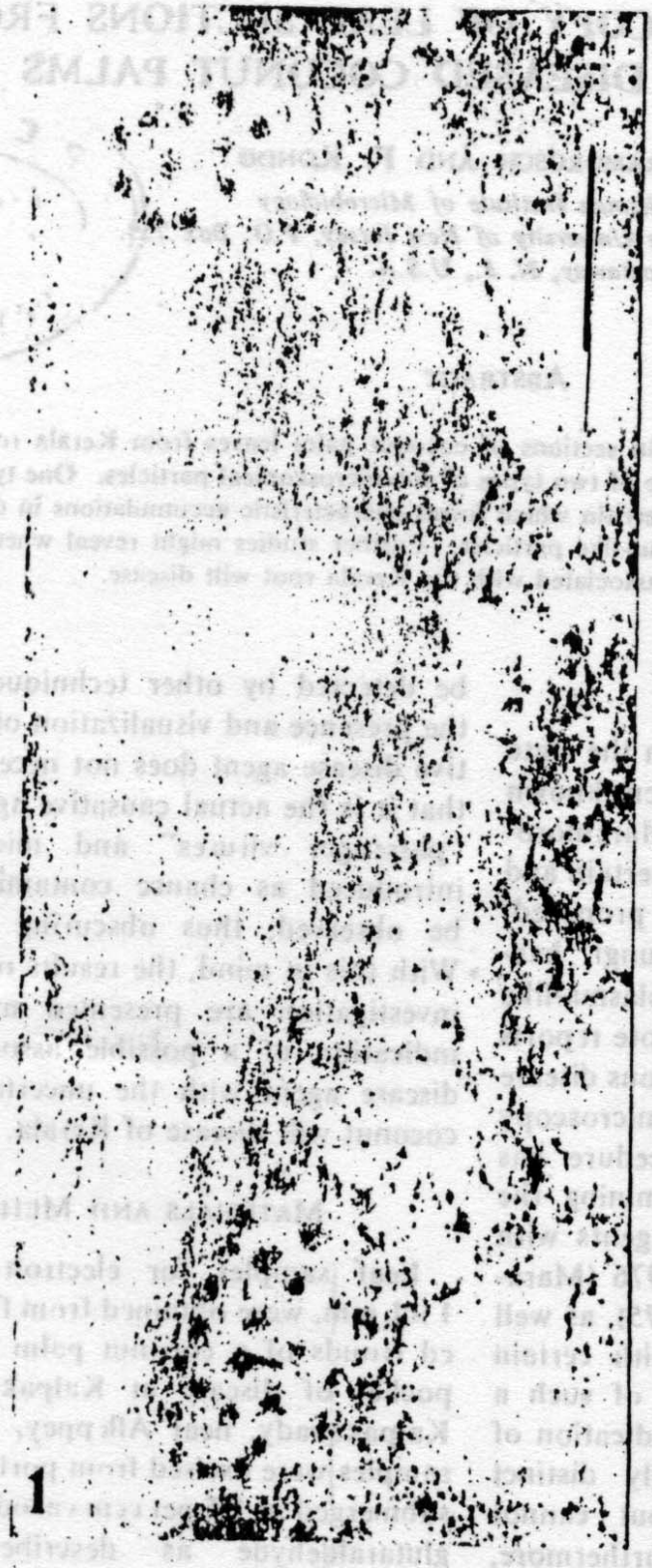

Fig. 1. A part of thin sectioned coconut palm cell from a diseased tree showing two accumulations of spherical, virus-like particles, 56 nm in diameter. The bar is equivalent to 500 nm. Magnification×53,000.

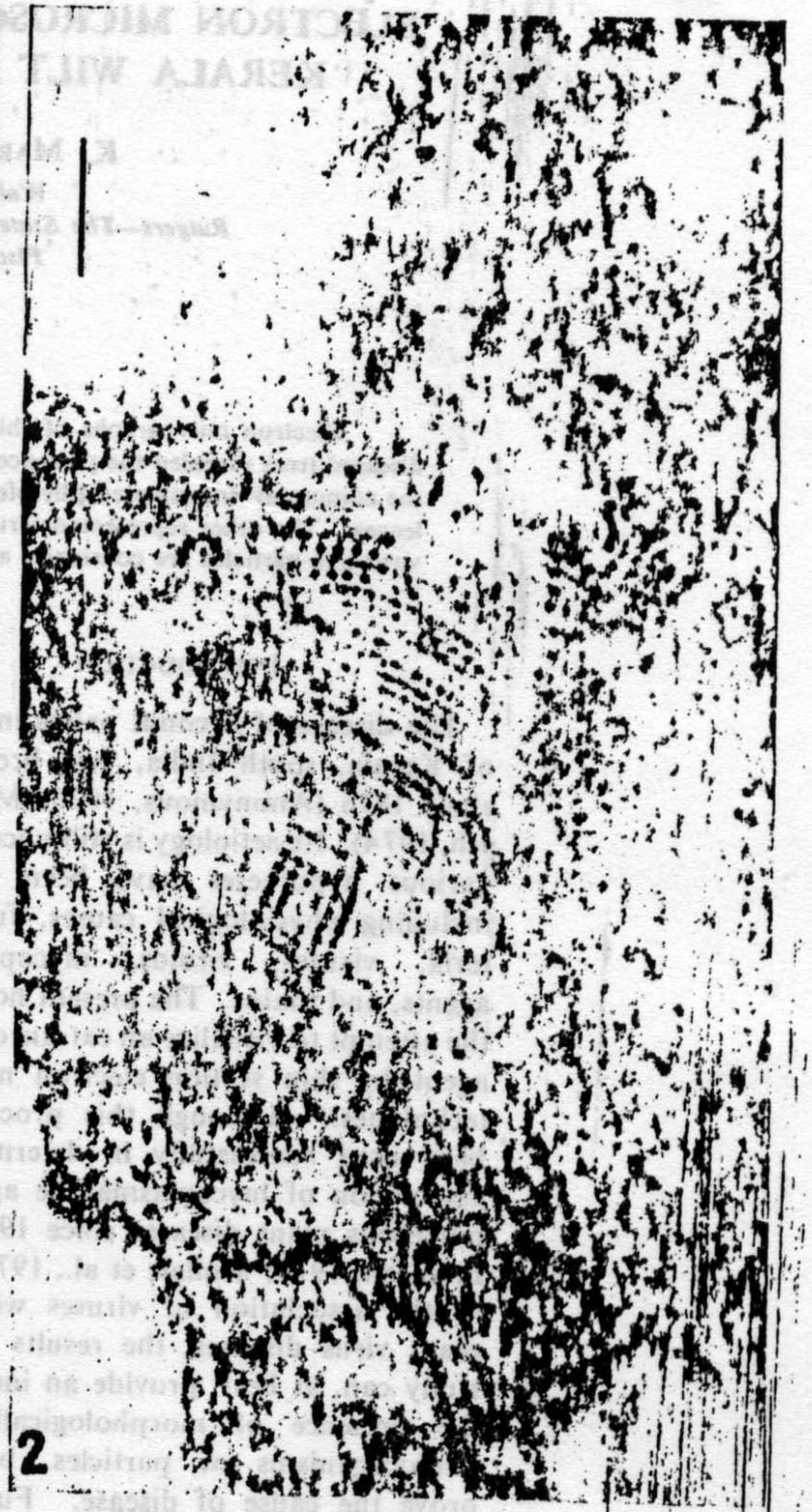

Fig. 2. Portion of chloroplast from a mesophyll cell from the same leaf as in Fig. 1. with phytoferritin particles in a typical crystalline array. The bar is equivalent to 100 nm. Magnification×180,000.

...cap so as to avoid the presence of an ...bubble. The vials were taken by air plane ...our laboratory and processed further 10 days later. Post-fixation in 2% buffered osmium tetroxide for 2 hr was followed by dehydration in a guarded ethanol series and by placement in propylene oxide. Infiltration with Epon 812 and embedding in this resin were followed by curing for 24 hr at 30, 45, and 60°C. Thin sections prepared on an MT-2 Sorvall ultratome, were examined with a JEM 120 electron microscope at 80 KV.

Results and Conclusions

In the sections of diseased leaf tissue icosahedral virus-like particles were occasionally observed in the epidermis and ground parenchyma cells. These particles appeared in small, isolated aggregates, as illustrated in Fig. 1. Since they were sparse and comparatively small, their detection was difficult. The approximate size of the particles was 56 nm in diameter. In addition to these virus-like particles, there were abundant clusters of electron-dense particles present in all diseased cells (Fig. 2). These structures appeared sometimes to form paracrystalline formations, varying in size and shape, but generally composed of spherules, 10 nm in diameter, forming a zig-zag pattern with angles of 120°. Individual particles were also observed in the vicinity of the crystal-like structures. The similarity in size and appearance of the spherules to those described in various diseased plants, and identified as phytoferritin (Kimura, Seveus, and Maramorosch, 1975; Maramorosch and Hirumi, 1973; Wildman and Hunt, 1976), led to the conclusion that in this instance also they represented phytoferritin accumulations. The crystalline aggregates were also visualized in unstained ultrathin sections, while the virus-like particles were not visible without staining with heavy metal (osmium tetroxide). This strengthened the conclusion that the sparsely occurring particles shown in Fig. 1 were of viral nature, while the numerous spherules occurring singly or in large aggregates represented accumulations of phytoferritin.

The above observations illustrate the possible association of an icosahedral virus with the coconut wilt disease. No similar particles have been described associated with either healthy plant material, or any of the known coconut palm diseases. Further extensive work will be required to establish whether the virus-like particles are constantly associated with diseased palms. If so, their nature and mode of transmission will be of special interest. The phytoferritin particle accumulations are common and their finding in diseased plants is to be expected not only in virus-infected material, but also in plants with nutritional defficiencies and plants infected with other disease agents. Their occurrence is presented here merely to call attention to the possible pitfalls in identifying such particles as "virus-like".

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