Phytoferritin Accumulations in Leaves of Diseased Coconut Palms

KARL MARAMOROSCH and HIROYUKI HIRUMI
Boyce Thompson Institute for Plant Research, Yonkers, New York, U.S.A.

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Summary
A study by electron microscopy of coconut palm (Cocos nucifera L.) leaves from trees infected by the Cape St. Paul wilt (Kaincopé) disease of West Africa was carried out. Samples were obtained during the dry season (Dec.-Jan.) and fixed immediately upon removal from the trees in buffered glutaraldehyde. Further processing for electron microscopy was carried out within a week. No virus particles, mycoplasma-like organisms (MLO), fungi, or bacteria were detected in thin sections. Crystalline or paracrystalline accumulations of electron-opaque granules, approximately 5.5–6 nm in diameter, were observed in disintegrated chloroplasts of mesophyll cells. Based upon their morphological characteristics, formation of the slightly curved, "fingerprint" arrays or linear rows running parallel, and the visualization of electron-opaque cores in unstained preparations, the granules were identified as phytoferritin particles.

1. Introduction
Crystalline arrangements of small particles of unknown nature in thin sections of diseased plants or infective insect vectors have often been considered as viruses (ALLEN 1972, CRONSHAW et al. 1966, ENGELBRECHT and ESAU 1963) or referred to as virus-like particles (GRANADOS 1969, LEE 1965, MOERICKE 1963, PARRISH and BRIGGS 1966). Sometimes crystalline arrays of phytoferritin macromolecules, non-viral protein containing iron particles, were observed but misinterpreted as virus particles (CRONSHAW et al. 1966, ENGELBRECHT and ESAU 1963) or not identified (KIM and FULTON 1969, SCHNEPF 1961, SECKBACH 1972, SITTE 1958, 1961). Phytoferritin, first identified microscopically, centrifugally, and spectrophotometrically in embryo extracts from pea by HYDE et al. (1962), was localized in proplastids in plastids of young bean leaves (HYDE et al. 1963). Phytoferritin particles have since been found...
in various plant tissues not involved in active photosynthesis and the subject has been reviewed by Seckbach (1972), who also demonstrated the accumulation of phytoferritin in plants experimentally overloaded with iron. The physical characteristics of the iron-protein ferritin from a species of Phycomyces have been described recently (David and Easterbrook 1971) and, in general, it is agreed that ferritin in plant and animal tissues plays a role as a storage for iron in a non-toxic form in the cell (Barton 1970).

Phytoferritin particles consist of iron-rich cores, 5.5–6 nm in diameter and spherical protein shells approximately 10.5 nm in diameter and electron lucent. The arrangements of the phytoferritin in thin sections of plants consist of irregular, paracrystalline, or crystalline arrays, often forming characteristic fingerprint-like clusters (Barton 1970, David and Easterbrook 1971, Seckbach 1972). The electron opacity of the iron-rich cores in thin section permits their visualization in electron micrographs without heavy metal staining and this provides a distinction between them and viruses or ribosomes (Barton 1970, Esau 1968, Robards and Humpherson 1967).

Phytoferritin has not been reported in plants with yellows-type diseases although the photosynthetic capacity of such plants is apparently reduced. This paper reports electron microscope observations of large accumulations of phytoferritin particles in coconut palm leaf material from trees affected by the Cape St. Paul wilt (Kaincopé) diseases of Ghana and Togo (Frémont et al. 1966, Maramorosch 1964). No similar accumulations were found in healthy appearing leaves.

2. Materials and Methods

Leaves from diseased coconut palms were removed immediately after felling the trees. Portions of the leaves, 1 × 2 cm, were excised and placed in 70% ethyl alcohol for 20–30 seconds, then immersed in 1.5% glutaraldehyde with 0.1 M sodium cacodylate buffer (pH 7.3), and cut into small pieces, approximately 1 mm in length and width. Further fixation and preparation for electron microscopy followed the procedure described elsewhere (Hirumi and Maramorosch 1972). Unstained sections were also prepared following the same procedures except omitting the uranyl acetate and lead staining. Both stained and unstained sections were examined using a Siemens Elmiskop-I electron microscope at 80 KV.

3. Results

Stained preparation. Disintegration of the plastid ultrastructure was observed in the mesophyll cells of yellowed regions. Limiting membranes (unit membranes) of the plastids were often ruptured. The membranous structure of the grana and stroma lamellae was disintegrated. Stromal materials were loosely dispersed. Ribosomes, as well as thylakoids, in the plastid stroma were indistinguishable. Large starch grains often accumulated in the plastids.

Many deformed plastids were found to contain paracrystalline or crystalline inclusions, composed of highly electron-opaque particles (Fig. 1). The particles