Mycoplasmalike Organisms Associated with Lethal Yellowing Disease of Palms

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ABSTRACT

Polymorphic, mycoplasmalike organisms were present in the sieve elements of young inflorescences of coconut, pritchardia, and veitchia palms affected by the lethal yellowing disease. The microorganisms were confined to recently matured protophloem and early metaphloem elements, and were rarely present in mature late metaphloem sieve elements. The organisms apparently moved from one sieve element to another through the sieve-plate pores along with the assimilate stream. The similarity of diagnostic symptoms of lethal yellowing in coconuts, pritchardias, and veitchias, and the occurrence of mycoplasmalike organisms in the sieve elements of all the three diseased palms during the earliest symptom of the disease, not only suggest that the three are affected by the same disease, but also suggest a mycoplasmalike etiology for lethal yellowing. This is the first detailed report of mycoplasmalike organisms in the phloem of diseased palms in Florida.

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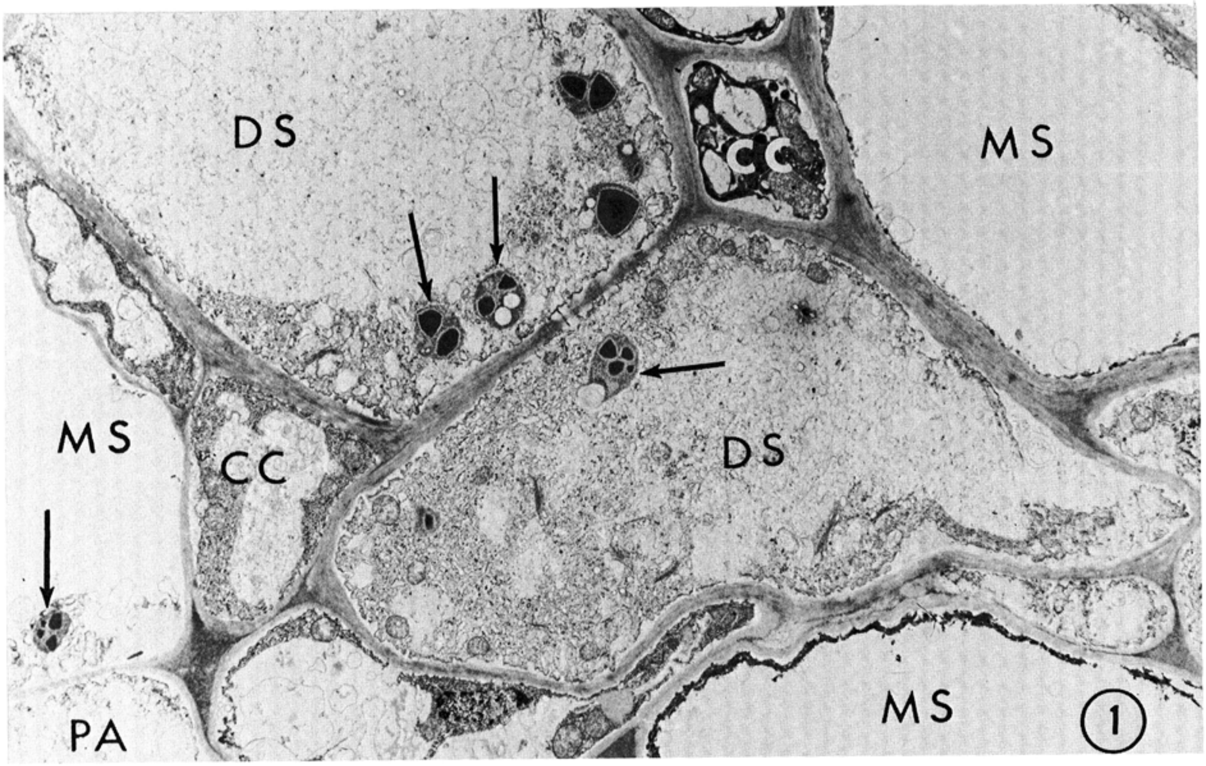
A disease of coconuts (*Cocos nucifera*) that is presently referred to as "lethal yellowing" was first reported by Fawcett (9) in Jamaica. It was later reported in other tropical regions including Cuba (2), the Bahama Islands, and the Dominican Republic (4), and in several regions in Africa (3, 10, 14). The disease has devastated coconut plantations in these countries and has brought tremendous economic loss to many of them. In the United States, the disease was first noticed in Key West in 1955 (7). Since then, it has spread quickly to other parts of southern Florida and has destroyed thousands of coconut palms. A recent study on the extent of the disease in the Miami area has revealed its apparent spread to two other palms – the popular Christmas palm (*Veitchia merrillii*) and a fan palm, *Pritchardia pacifica* (18). The symptoms of the disease in affected coconut palms have been extensively described by several workers (5, 6). The diseased pritchardias and veitchias also exhibit symptoms that are very similar to those of coconuts affected by lethal yellowing (18).

Recently Beakbane et al. (1), and Plavsic-Banjac et al. (20), reported mycoplasmalike organisms (MLO) in the phloem of coconut palms (cv. 'Jamaican Tall') affected by lethal yellowing in Jamaica. This paper presents additional information on the occurrence of MLO in the phloem of coconut palms affected by lethal yellowing, and the first detailed report on the occurrence of MLO in that of pritchardias and veitchias suspected of having the same disease. For a general account of the problem of classification and nomenclature of mycoplasmas and mycoplasmalike organisms, the reader is referred to Maramorosch et al. (13), and Davis and Whitcomb (8).

MATERIALS AND METHODS.—The coconut palm material (*Cocos nucifera* L. cv. Jamaican Tall) was collected both in Jamaica and Florida. The Jamaican material was collected on 4 January 1970 at the Kildaire Research Station, Jamaica, and the Florida material on 23 May 1973 in southwest Miami. Two palms from the Kildaire Research Station, and two from southwest Miami showing early symptoms of lethal yellowing were felled, and parts of young and old leaves, and expanded and unexpanded inflorescences (including those that had 20-50% necrosis at the distal ends of rachillae) were collected. In addition, samples from various parts of the stem were collected. Healthy material of the same variety was collected in 1971 at the A. Jennings Estate, Coral Gables, Florida, for comparison. Similar material of diseased and healthy *Pritchardia pacifica* Seem. & H.



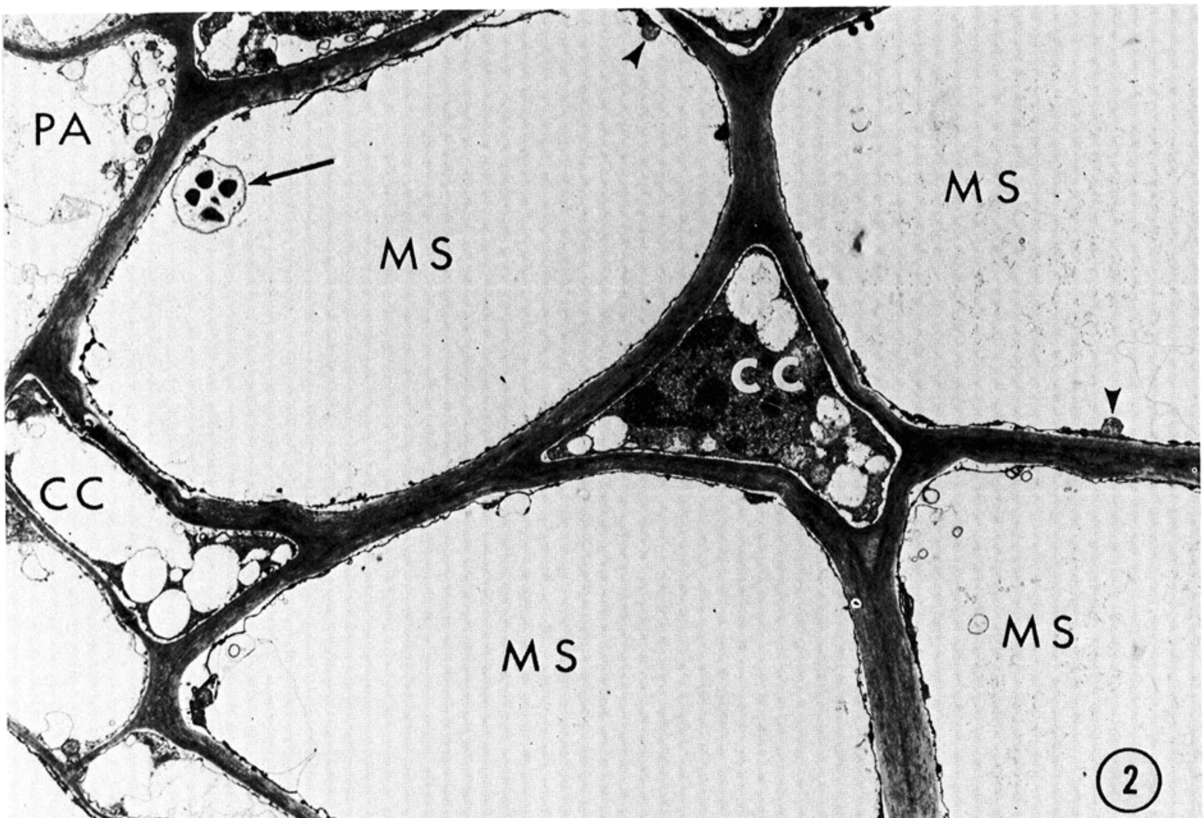
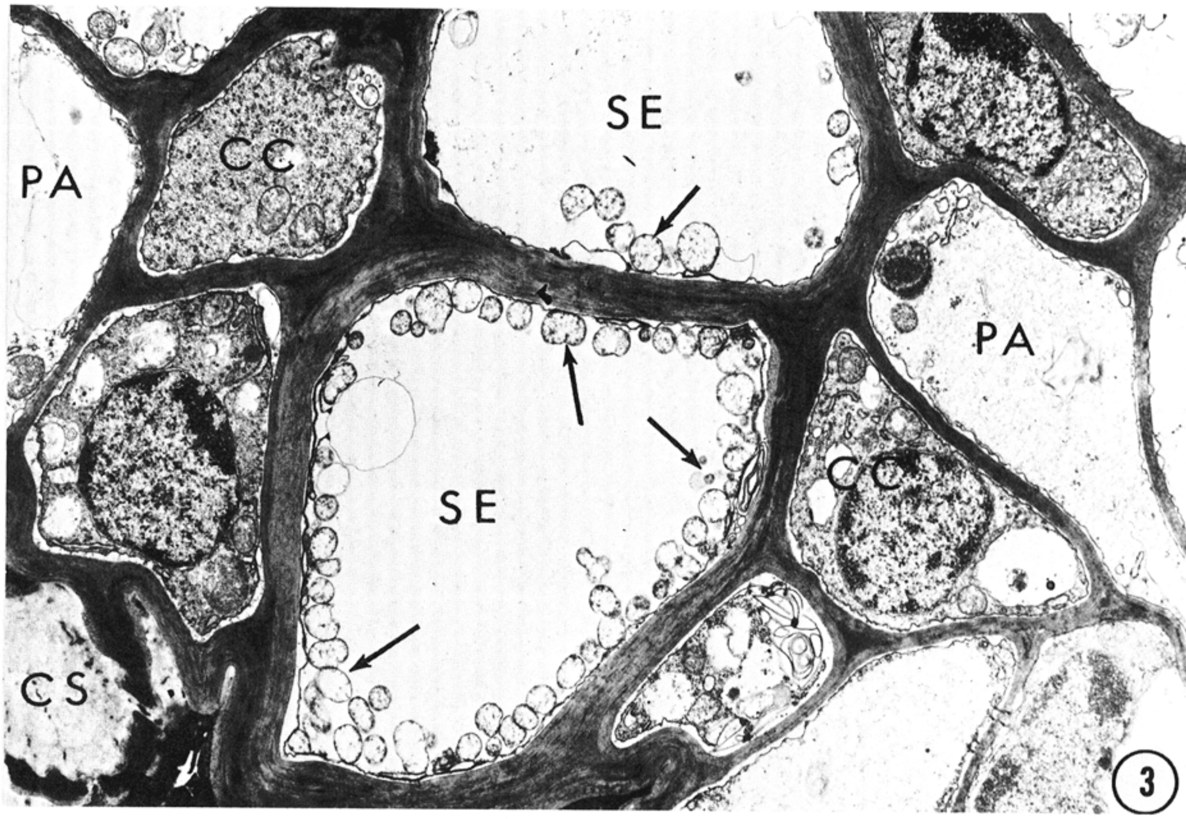


Fig. 1 and 2. Transverse sections of differentiating, and mature metaphloem sieve elements, respectively. **1**) Differentiating (DS), and recently matured late metaphloem sieve elements (MS) in the rachilla of *Pritchardia pacifica* affected by lethal yellowing. Mycoplasmalike organisms are not present in these late metaphloem cells. Arrows indicate sieve-element plastid with cuneate crystals and starch granules. CC = companion cell; PA = parenchyma cell (× 8,000). **2**) Mature, late metaphloem sieve elements (MS) in an expanded inflorescence axis of *Cocos nucifera* (Jamaican material) affected by lethal yellowing, showing the absence of mycoplasmalike organisms in the elements. The sieve-element plastid (arrow) and mitochondria (darts) are relatively intact. CC = companion cell; PA = parenchyma cell (× 6,500).



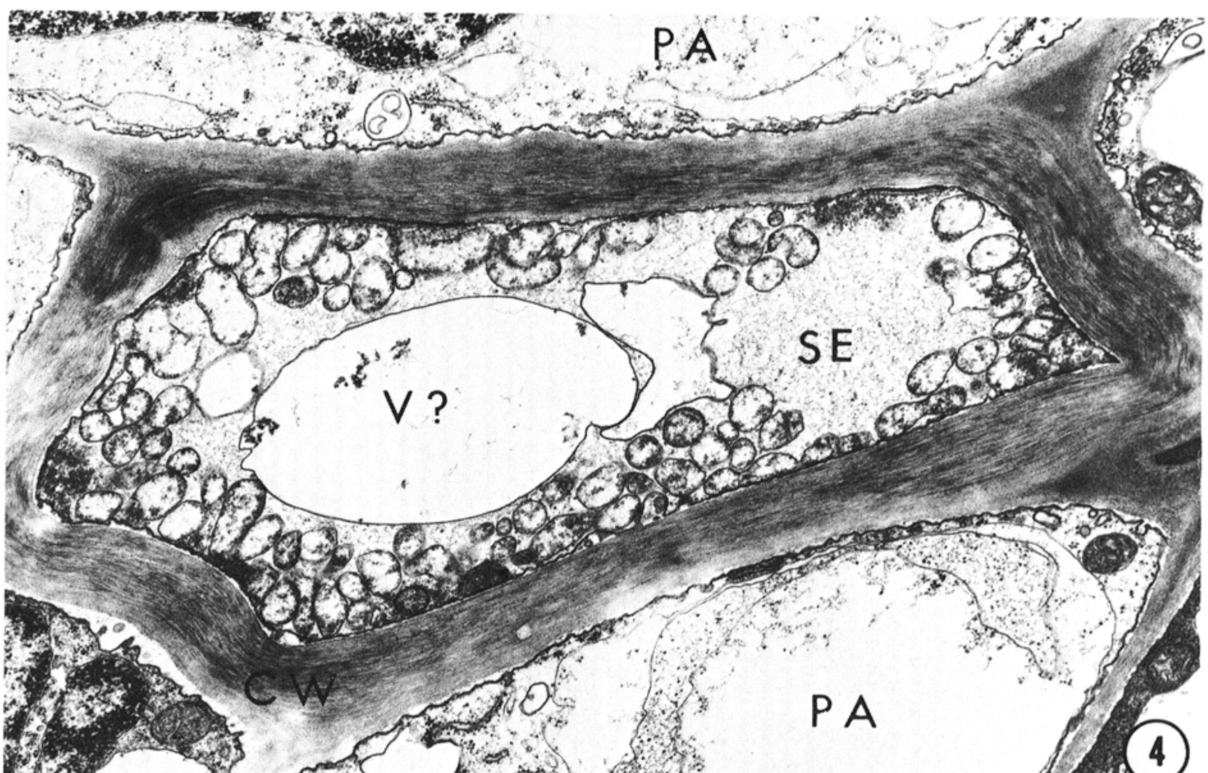


Fig. 3 and 4. Transverse sections of recently matured sieve elements of a young inflorescence axis of *Cocos nucifera* (Jamaican material) affected by lethal yellowing disease. **3)** Two relatively undisturbed, recently matured early metaphloem elements (SE) containing mycoplasmalike organisms (arrows). Note the parietal distribution of the mycoplasmalike organisms in the sieve elements. Crushed protophloem elements (CS), companion cells (CC) and parenchyma cells (PA) are also visible (× 9,000). **4)** A recently matured protophloem sieve element (SE) containing mycoplasmalike organisms. The nacreous cell walls (CW) and the presumably disrupted vacuole (V?) suggest that the element had matured recently. PA = parenchyma cell (× 15,000).

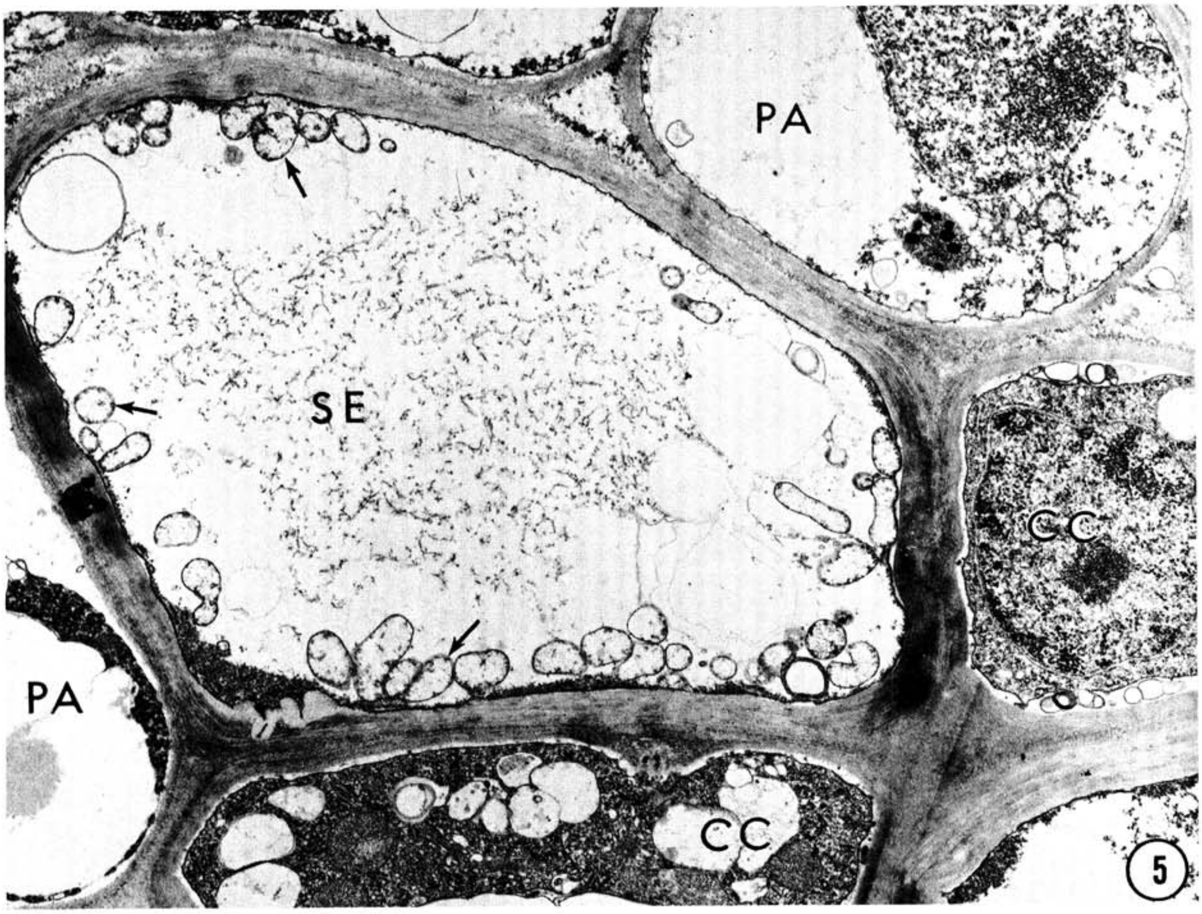


Fig. 5. Transverse section of early metaphloem elements of a young inflorescence axis of *Veitchia merrillii* affected by the disease, showing mycoplasmalike organisms (arrows) in a mature sieve element (SE). CC = companion cell; PA = parenchyma cell (× 14,000).

Wend. and *Veitchia merrillii* (Becc.) H. E. Moore was collected in southwest Miami on 23 April 1973 and 23 May 1973, respectively.

The material was fixed in either formaldehyde/glutaraldehyde (11), or in 5% glutaraldehyde in 0.05-0.1 M cacodylate buffer, followed by post-fixation in buffered 2% osmium tetroxide. Remaining preparation for electron microscopy was similar to that described by Parthasarathy and Muhlethaler (19).

RESULTS.—The results described here are true for each of the three species of palms which were observed, unless otherwise stated. MLO were found in mature protophloem sieve elements and in recently matured metaphloem sieve elements of diseased palms taken from the region of the rachilla (flowering axis) just below the necrotic parts of unexpanded inflorescences that were still enclosed in the bracts. Sieve elements of partly expanded spear leaves had some MLO, but never as much as those in young rachillae. No MLO were found in differentiating or mature late metaphloem elements of fully expanded inflorescences, leaves, or stems of the affected palms (Fig. 1 and 2). MLO were also absent in the mature sieve elements of protophloem and metaphloem in all organs of healthy palms.

When present, the microorganisms were always confined to mature sieve elements and never occurred in the parenchymatic elements (Fig. 3-5). The MLO were polymorphic and varied from 0.4 μ to 2 μ in size, depending on the form and the plane of sectioning. The ovoid forms in relatively undisturbed sieve elements ranged from 0.6-0.8 μ in diam. The MLO were bounded by a typical triple-layered membrane about 80 Å thick (Fig. 6-10). The internal structure of the MLO closely resembled that described by Plavsic-Banjac et al. (20) and Beakbane et al. (1). In well-fixed preparations, the central part of the microorganisms had a network of fibrillar material about 25-30 Å in diam. The network was surrounded by a slightly granular, peripheral zone that normally contained ribosome-like particles (Fig. 6-10). Serial sections often revealed MLO that appeared to be undergoing binary fission (Fig. 6, 9). Several, small, oval or spherical, electron-dense bodies 0.1-0.2 μ in diam were also often present along with the polymorphic bodies (Fig. 8). Such dense bodies closely resembled the "elementary bodies" described by several workers (cf. 13).

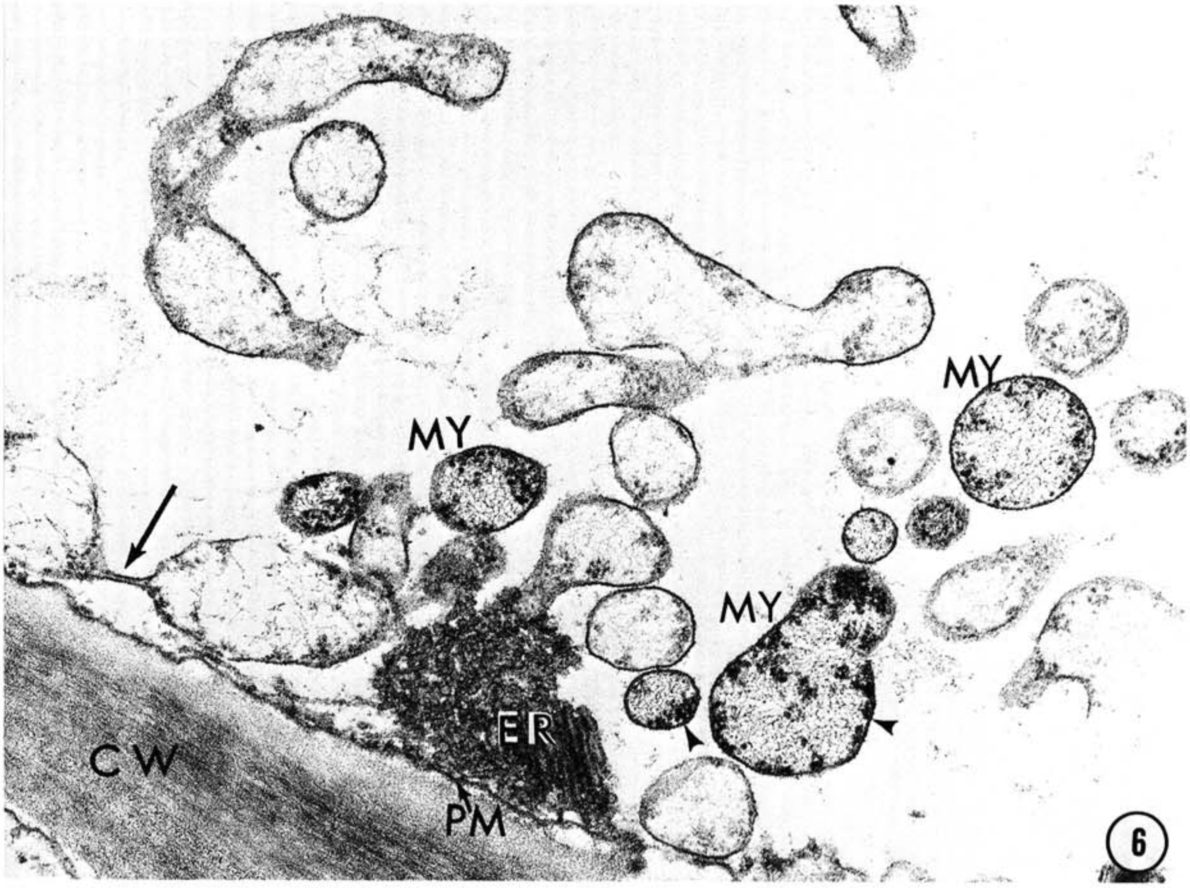
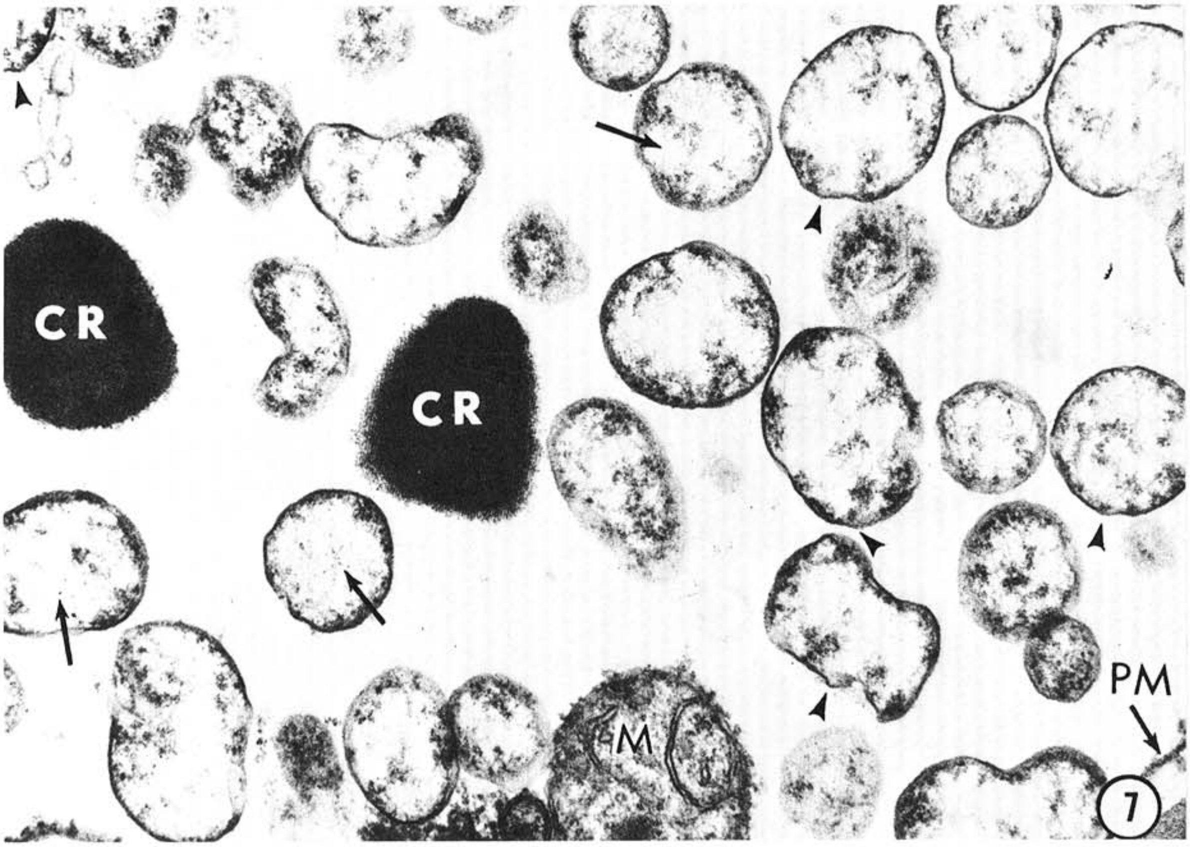


Fig. 6. Mycoplasmalike organisms (MY) in a mature, early metaphloem sieve element of a young inflorescence axis of *Cocos nucifera* (Florida material). The triple-layered unit membrane (darts) and the central fibrillar network are well preserved in some of the organisms. One of the organisms is apparently undergoing binary fission (arrow). CW = cell wall; ER = endoplasmic reticulum; PM = plasmalemma (× 49,000).

Not all the vascular bundles in an infected organ contained MLO, and not every sieve tube in an infected bundle had the microorganisms. Preliminary investigations suggest that the structure of sieve elements that contained MLO did not usually differ appreciably from the elements that lacked them. There are several exceptions, however. Some sieve elements that contained MLO also had a fine fibrillar and/or amorphous substances in the cell lumen (Fig. 4, 10). Morphologically these substances did not resemble the P-protein normally present in some palms (15, 17). In addition to the relatively intact mitochondria (Fig. 7), sieve elements that had MLO also contained membranes of unknown origin (Fig. 3, 4, 5, 9). Such membranes were single, double or multi-layered and usually stained darker than plasmalemma or other unit-membranes of normal cells (Fig. 4, 9). Vesicular and tubular ER that is normally present in abundance in recently matured protophloem elements of healthy tissues (16, 17) was scarce in elements that contained MLO. Sieve-plate pores of infected sieve elements were lined with callose, and several thin-sections where the MLO were apparently passing through the pores were obtained from the Jamaican and Floridian material of *Cocos nucifera* (Fig. 8). Slender, stretched forms of MLO 400-600 Å in diam at the narrowest parts, were more often present in and near the sieve-plate pores than elsewhere in the sieve element. In relatively undisturbed sieve elements, the MLO occurred at the parietal position of the cell, often in contact with plasmalemma (Fig. 3 and 4). The MLO in such sieve elements almost always had an oval or slightly elongated form and rarely the slender form (Fig. 3). Some mature sieve elements that contained MLO had invaginations of plasmalemma superficially resembling the MLO. The invaginations often contained fibrillar material comparable in size with that inside the MLO (Fig. 10 - unlabelled arrows). However, such invaginations, which are common in many healthy palm sieve elements (15), lack ribosome-like structures and can thus be readily distinguished from the MLO (Fig. 10).

DISCUSSION.—The MLO present in the phloem of coconut, pritchardia, and veitchia palms affected by lethal yellowing disease are very similar in appearance to MLO found in other diseases of the yellows type (13). It is not clear whether the polymorphism exhibited by the MLO in the present investigation is real or an artifact. Distortion or disruption of organelles in sieve elements of palms during fixation is a common phenomenon (17). It is



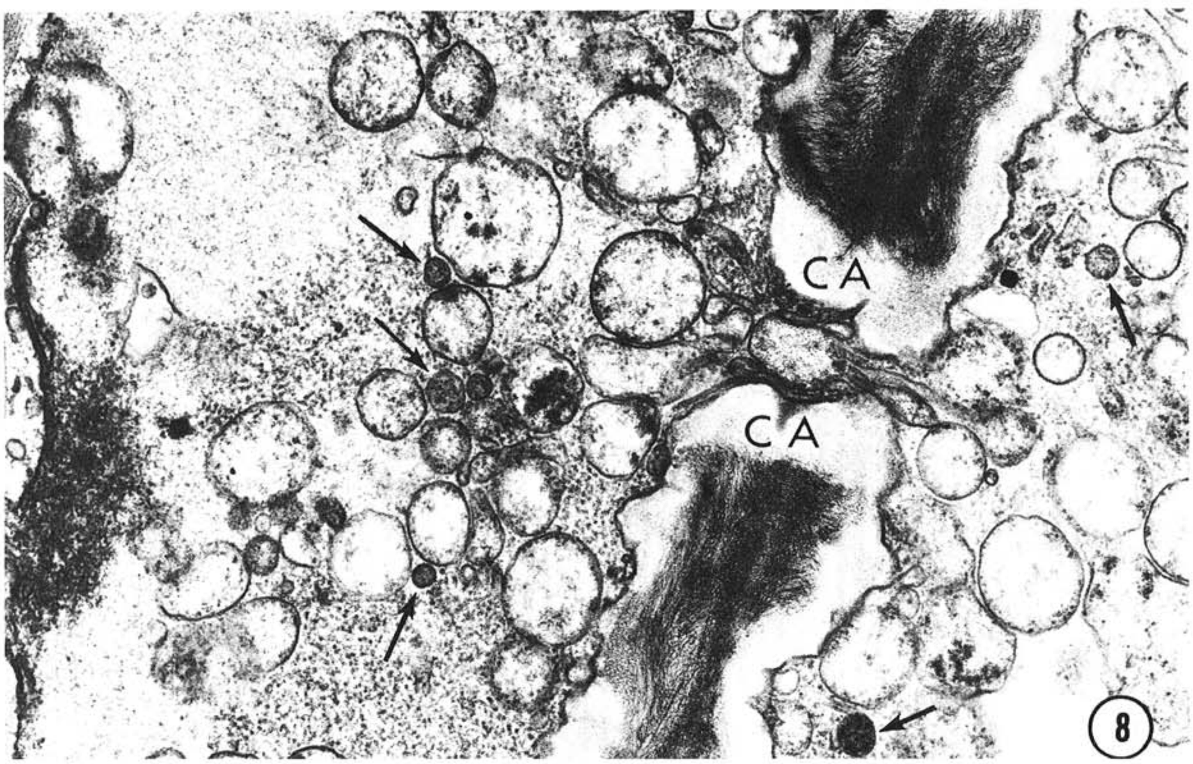


Fig. 7 and 8. Mycoplasmalike organisms in mature sieve elements of young inflorescence axis of *Cocos nucifera* (Jamaican material). **7)** A magnified view of mycoplasmalike organisms in mature protophloem sieve element. The bodies are bound by a unit-membrane (darts) and contain a fibrillar network in the center (arrows). A relatively intact mitochondrion (M) and two cuneate crystals (CR) that presumably were displaced from plastids during fixation, are also visible. PM = plasmalemma (× 38,000). **8)** Mycoplasmalike organisms apparently passing through a sieve-plate pore lined with callose (CA). Note the plasticity of the bodies in the pore. Arrows indicate smaller, electron-dense vesicles comparable to "elementary bodies" (× 25,000).

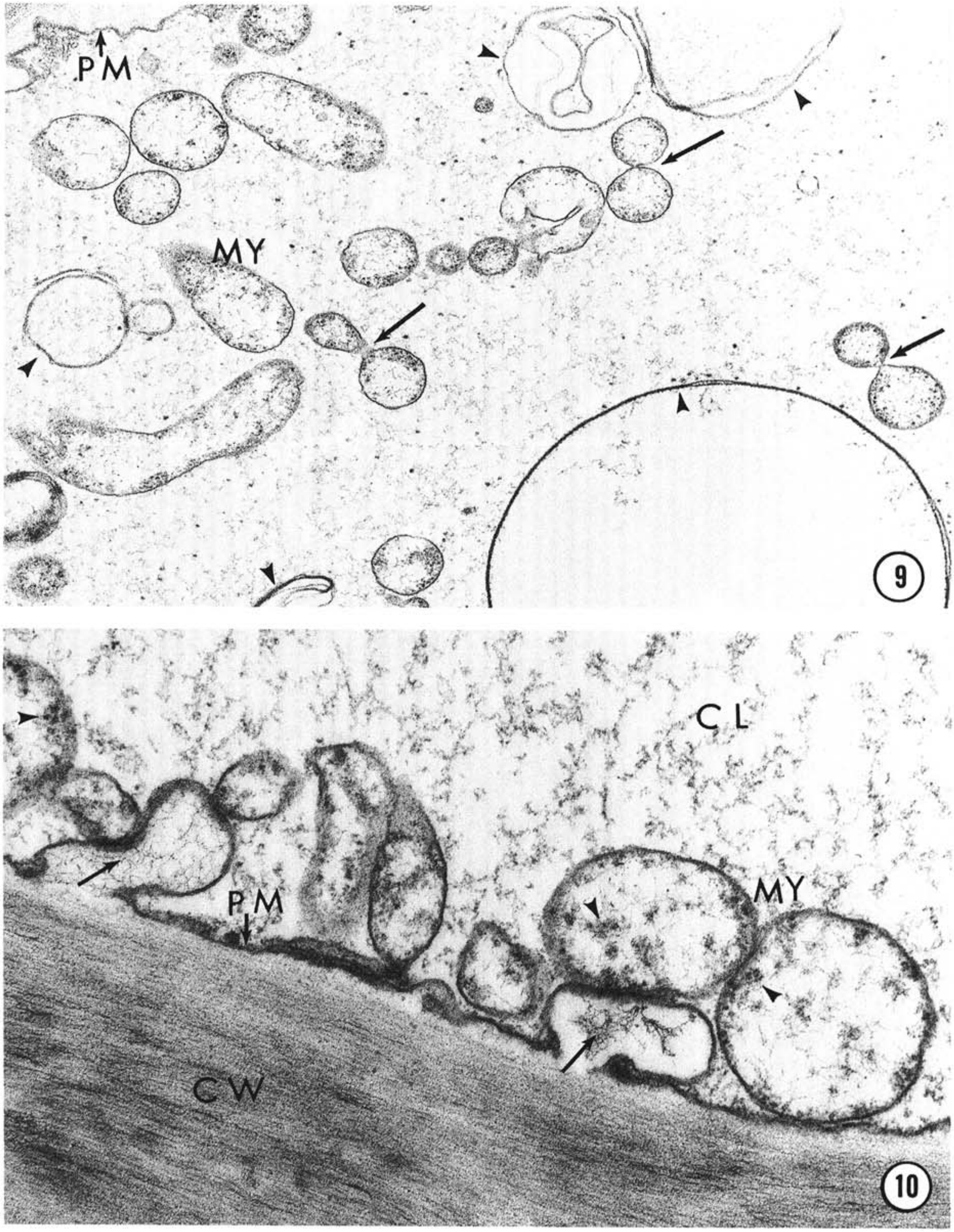


Fig. 9 and 10. Mycoplasmalike organisms in recently matured metaphloem sieve elements of young rachillae of *Pritchardia pacifica*. **9)** Several mycoplasmalike organisms (MY) are apparently undergoing binary fission (arrows). Darts indicate membraneous structures of unknown origin. Note the fine, fibrillar substance present in the sieve-element lumen. PM = plasmalemma (× 40,000). **10)** Same as Fig. 9. Note the invaginations of plasmalemma that contain fibrillar material (unlabeled arrows) but not the ribosome-like material (darts) that are present in the mycoplasmalike bodies. CL = cell lumen; CW = cell wall; PM = plasmalemma (× 60,000).

thus likely that some of the elongated or filamentous forms are artifacts created by pressure release in sieve elements at the time of fixation. The rare occurrence of intact plastids in sieve elements that contain highly elongated or filamentous MLO further suggests that disturbance of sieve elements during fixation might be responsible for the polymorphism.

The predominant occurrence of MLO in mature protophloem sieve elements and early metaphloem sieve elements, and the apparent absence of MLO in the matured late metaphloem sieve elements of expanded leaves and inflorescences suggest, that, the microorganisms had moved along in the sieve tubes with the assimilate stream to the "sink" regions such as young inflorescences and young leaves. The passage of MLO through the sieve plate pores in functioning sieve elements should pose no problem since the pores in palms are probably open in vivo (17). The relatively normal appearance of sieve elements containing MLO, with the exception of some and the ability of MLO-containing elements to synthesize callose lead one to speculate that the presence of these organisms does not drastically affect the functioning of at least the recently matured sieve elements. A knowledge of the exact stage of sieve element development at which the MLO gain entry into the element would be of considerable interest. Although the present investigation is inadequate to draw conclusions on this aspect, the absence of MLO in immature sieve elements with undifferentiated pores suggest that the sieve plate pores may have to be at least partially formed before the microorganisms can pass into the sieve element. The extreme plasticity of the MLO demonstrated in Fig. 8 indicates that they may be able to pass through pores $\pm$ 600 Å in diam. Furthermore, the ability of mycoplasmas to pass through filter pores that are considerably smaller than the diam of the microorganisms has been well demonstrated (12, 21). Thus the sieve plate pores need not be fully differentiated (i.e., up to a diam of 1-2 μ) before the MLO are able to pass through the pores.

The similarity of diagnostic symptoms of lethal yellowing in coconuts, pritchardias and veitchias, and the occurrence of MLO in the sieve elements of all the three species of diseased palms strongly suggest that the three are affected by the same disease. Furthermore, the absence of other pathogens in the earliest symptoms of the disease suggest that the MLO may be causative agents of the disease. Temporary remission of the disease that has recently been achieved by tetracycline chemotherapy on affected coconut palms (D. H. Romney, Director of Research, Coconut Industry Board, Jamaica, and R. E. McCoy, Assistant Professor, Agriculture Research Center, Ft. Lauderdale, Florida - *personal communications*) adds credence to MLO as the possible causative agents of lethal yellowing. Plavsic-Banjac et al. (20) have also suggested the possibility of a mycoplasmalike etiology for coconut lethal yellowing. However, the same authors have pointed out the need for more studies on the etiology of the disease in order to determine the causal agents beyond any doubt. Obtaining unequivocal proof of a mycoplasm etiology for lethal yellowing will be more difficult than might be imagined because of the lack of knowledge concerning the vector of the disease, the difficulty of maintaining yellows agents in cell-free media (8), and the basic deficiencies in understanding of the morphology, structure, and physiology of palms.

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