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THE USE OF ELECTRON MICROSCOPY FOR LETHAL YELLOWING DIAGNOSIS[1]

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Abstract. **Symptoms of the lethal yellowing (LY) disease on mature coconut palms are distinct and can be used for LY diagnosis. The symptomatology of LY on younger, non-fruiting coconut palms and on palm species other than coconut, frequently lacks the specificity for positive LY identification; in these cases disease confirmation has been determined by surveying phloem tissue with an electron microscope for mycoplasmalike organisms (MLO). The effectiveness of LY diagnosis by electron microscopy is limited by 1) apparent absence of MLO from most mature palm tissue, 2) the low concentration of MLO in most palm species, 3) the unpredictable localization of MLO, and 4) the small sample size that can be examined. Despite the limitations of electron microscopy, no other reliable means of LY diagnosis is available. This method has been used to ascertain MLO infection in 31 palm species and it is routinely employed on palms utilized in disease transmission trials.**

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Lethal yellowing (LY), a disease that has been destructive to coconut palms (*Cocos nucifera* L.) in the Greater Antilles, the Bahamas and Florida, has received extensive research effort since the beginning of this century. Early investigations on LY were rigorous and focused on fungi, bacteria, viruses, nematodes, insects, and nutritional disorders as possible causes. Despite the intensity of this research, the LY pathogen eluded discovery until 1972 when laboratories in three separate countries reported the presence of mycoplasmalike organisms (MLO) within the phloem vascular tissue of coconut palms infected with LY (1, 3, 8).

Mycoplasmalike organisms are too small to be identified with a light microscope, and they went unrecognized as plant pathogens until 1967 when Japanese scientists first observed them in diseased plants with the aid of an electron microscope (2). Since that first discovery, electron microscopic studies have attributed the cause of over 100 plant diseases to MLO infection. In order to be classified as a true mycoplasma, an organism must first be grown on culture media and then undergo an array of diagnostic tests for growth requirements and serological affinities to other mycoplasmas. Because the MLO associated with most plant diseases, including LY, have defied cultivation attempts, these organisms must be called "mycoplasmalike" for the present.

Both mycoplasmas and MLO have internal contents,

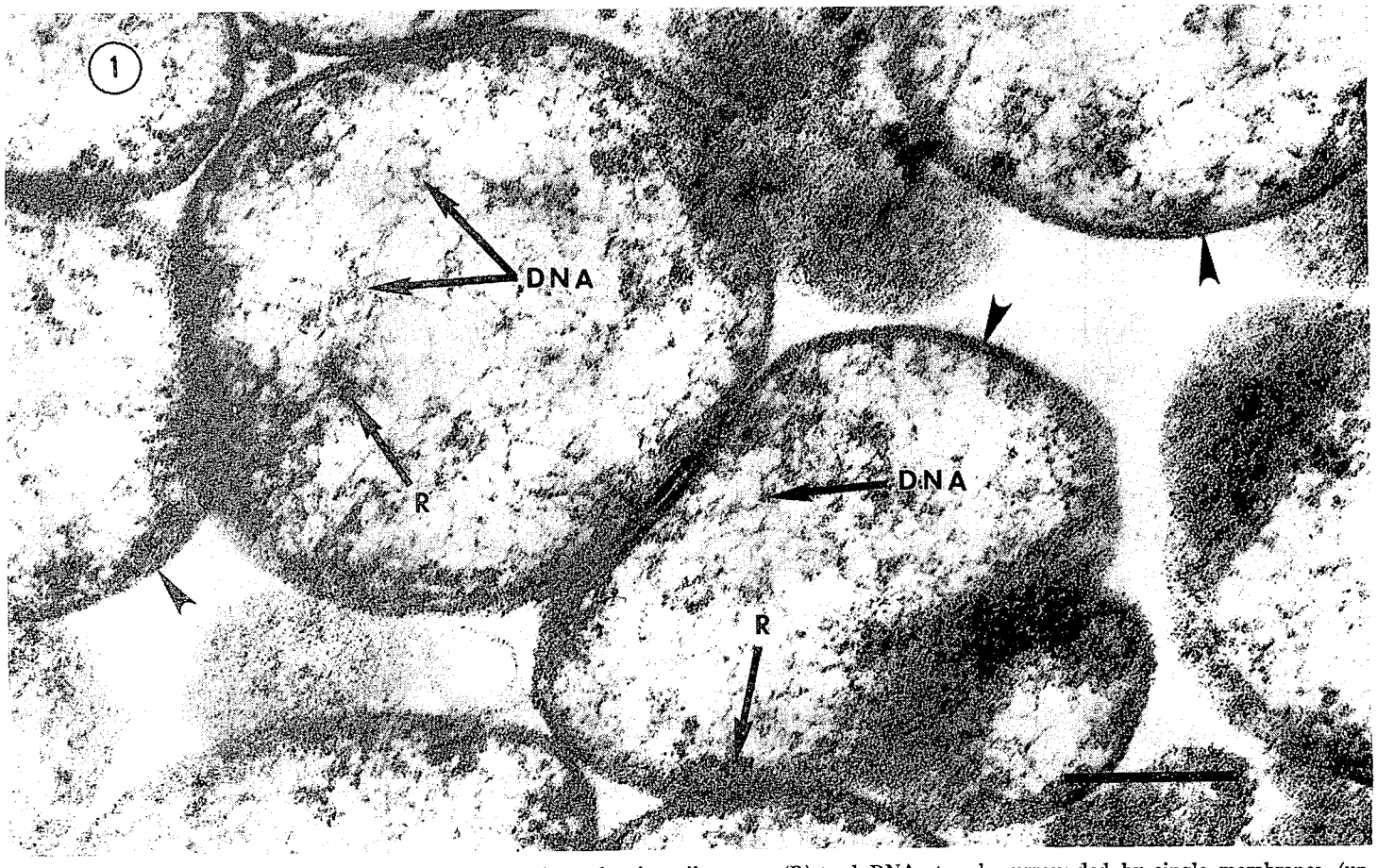

Fig. 1. High magnification of mycoplasmalike organisms showing ribosomes (R) and DNA strands surrounded by single membranes (unlabeled darts). Bar = 0.1 μm.

such as ribosomes and genetic material (DNA), that resemble the contents of bacteria, but they lack the inflexible cell walls that surround bacterial bodies (Fig. 1). Consequently, they may assume a variety of shapes from spherical to filamentous (Fig. 2). Mycoplasma and MLO are sensitive to tetracycline antibiotics which destroy bacterial ribosomes, but they are insensitive to antibiotics such as penicillin which affect bacteria by attacking their rigid cell walls. The theraputic value of oxytetracycline and the ineffectiveness of penicillin in the treatment of LY has been well documented (4, 5). This antibiotic response of LY, together with the consistent association of MLO in LY-diseased palms, form the strong scientific basis to conclude that MLO are the cause of LY.

The symptoms of LY on mature, bearing, 'Jamaica Tall' coconut palms are diagnostic for the disease. They include premature shedding of all fruit on the tree, the discoloration and death of emerged and unemerged flower spikes (inflorescences), a yellowing of older and then younger leaves, and frequently a soft rot within the crown that precedes the death of the palm. Because non-bearing coconut palms affected by LY lack fruit and flower symptoms, it is impossible to diagnose LY conclusively in these palms by symptom expression only. Therefore, electron microscopic examination for MLO presence is needed for LY verification.

Following the outbreak of LY on the Florida mainland in 1971 and the associated epidemic loss of coconut palms, unusual losses of other palm species were also noted in areas where LY was severe. Lethal yellowing was suspected in these additional palm species, but the problem of LY diagnosis was complicated by two factors: many of the palms were not in reproductive cycles and, therefore, could not show fruit or flower symptoms, and some of the affected species did not show the characteristic yellow leaf color of affected 'Jamaica Tall' coconut palms. The only other means to diagnose LY was to determine whether the *dying palms* contained MLO as did diseased coconut palms. Initial electron microscope studies did identify MLO infection in dying members of two popular palm species, the Christmas palm, *Veitchia merrillii* (Becc.) H. E. Moore and the Fiji Island fan palm, *Pritchardia pacifica* Seem. & H. Wendl. (7). Therefore an intensive electron microscopic investigation was initiated by the University of Florida to estimate the potential threat of LY to the many other palm species grown in Florida. This paper summarizes this study, portions of which have been published in greater detail elsewhere (9).

Materials and Methods

Information on the location of dying palms other than coconut was solicited from the Florida Department of Agriculture's Division of Plant Industry, the University of Florida's County Extension Offices and Fairchild Tropical Garden, as well as from members of the Palm Society and citizens of South Florida. The decline of some palms that were reported was attributed to causes other than LY. Butt rot caused by *Ganoderma* infection and bud infestations by palm weevils were commonly encountered. When neither of these or other recognized causes of palm declines could be detected, the palms were sampled to determine whether they contained MLO. Whole palm crowns were collected in the field and brought to the laboratory where they were further dissected. Tissue samples were taken from young, unemerged leaf bases within 3 cm of the apical meristem

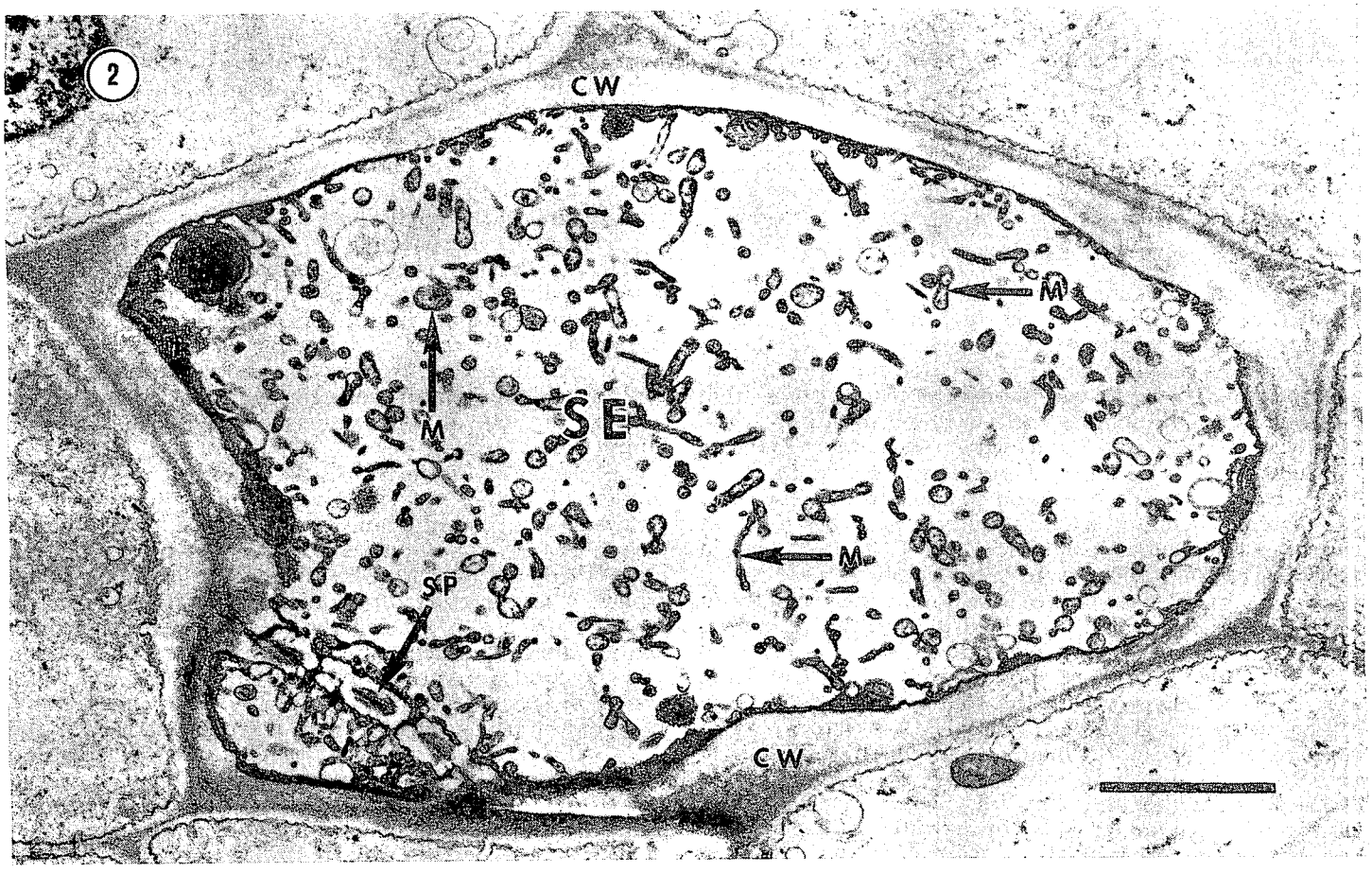

Fig. 2. Mycoplasmalike organisms (M) within a sieve element (SE) of *Borassus flabellifer*. CW = host cell wall; SP = sieve plate; bar = 3 μm.

and processed for electron microscopy as described previously (9). The number of diseased and healthy palms of each species examined is given in Table 1. The choice and number of palms studied was largely determined by the availability of suitable material. As far as possible, healthy control palms were collected from LY-free areas.

Table 1. Species of palms susceptible to lethal yellowing.

Palm species	Declining trees	Healthy trees
Allagoptera arenaria (Gomes) O. Kuntze	1/1[z]	—[y]
Aiphanes lindeniana (H. Wendl.) H. Wendl.	1/1	—
Arenga engleri Becc.	5/6	0/3
Arikuryroba schizophylla (Mart.) L. H. Bailey	1/3	0/1
Borassus flabellifer L.	2/2	—
Caryota mitis Lour.	3/6	0/2
Chrysalidocarpus cabadae H. E. Moore	2/4	0/2
Cocos nucifera L.	7/9	—
Corphya elata Roxb.	1/1	—
Dictyosperma album (Bory) H. Wendl. & Drude ex Scheff	2/3	0/1
Gaussia attenuata (O. F. Cook) Becc.	1/1	—
Howea belmoreana (C. Moore & F. J. Muell.) Becc.	1/1	—
Hyophorbe verschaffeltii H. Wendl.	1/2	—
Latania spp. Comm.[x]	2/2	—
Livistona chinensis (Jacq.) R. Br. ex Mart.	2/2	—
Nannorrhops ritchiana (Griff.) Aitch.	1/1	—
Phoenix canariensis Hort. ex Chabaud	4/4	—
Phoenix dactylifera L.	3/6	0/1
Phoenix reclinata Jacq.	1/3	0/5
Phoenix sylvestris (L.) Roxb.	1/1	—
Pritchardia affinis Becc.	1/1	—
Pritchardia remota Becc.	1/1	—
Pritchardia thurstonii F. J. Muell. & Drude	3/3	—
Pritchardia spp. Seem & H. Wendl.[x]	3/3	—
Ravenea hildebrandtii H. Wendl. ex Bouche	1/1	—
Trachycarpus fortunei (Hook) H. Wendl.	2/2	0/2
Veitchia merrillii (Becc.) H. E. Moore	9/12	0/9
Veitchia montgomeryana H. E. Moore	1/1	—
Total	63/83	0/26

[z]Number of palms where MLO were seen/number of palms examined.
[y]No examinations made.
[x]Identification made to genus only. Each palm a separate species.

Results

Mycoplasmalike organisms were observed in phloem tissue from 63 declining palms that included 31 species (Table 1). These organisms were never found in the 26 healthy palms that served as controls for this study. Early attempts to find MLO within mature palm tissues from emerged leaves and inflorescences were mostly unsuccessful. However, MLO were found with reasonable regularity within the immature bases of unemerged leaves from the same palms. The only exception to the low concentration or apparent absence of MLO in mature tissue was noted in two diseased coconut palms where MLO were seen in "flag" leaves, the first leaves to turn yellow.

Mycoplasmalike organisms were found most readily in the young leaf bases that contained the greatest concentration of functional sieve elements, and the success of finding MLO decreased sharply as progressively older leaf bases were examined. Even within the younger tissues the MLO concentration was generally low. No MLO were found in approximately one-fourth of the declining palms that were sampled. Many diseased palms had MLO in less than 5% of the vascular bundles, but infrequently samples were examined that contained MLO in over 50% of the vascular bundles. The numbers of MLO varied considerably among different plants, but some species such as the Canary Island date palm, *Phoenix canariensis* Hort. ex Chabaud, and the windmill palm, *Trachycarpus fortunei* (Hook) H. Wendl., contained considerably more MLO than other species such as the true date palm *Phoenix dactylifera* L. The MLO concentrations in coconut and Christmas palms were among the lowest of any species examined.

The MLO within the samples were not uniformly distributed but were normally found in relatively high numbers in isolated areas. In most instances, individual vascular bundles were either completely free of MLO or would contain the organisms in most of their sieve elements. Several of the diseased palms that had low MLO populations overall, actually had high numbers of MLO in a few vascular bundles so that many sections had to be examined before the organisms were found.

Discussion

Although healthy control palms were not studied for each species and the sample size for some species was small, the data as a whole strongly support the hypothesis that MLO are the organisms responsible for the death of the palm species examined in this study. Positive therapeutic responses to oxytetracycline by declining *Arikuryroba, Pritchardia* and *Trachycarpus* palms, and successful results in the preventive treatment of *Veitchia* palms with oxytetracycline (6), add additional support to this hypothesis. Furthermore, the similarity of symptom expression and the close chronological and geographical coincidence of the various palm declines with LY, indicate that all these palm maladies are caused by the same pathogenic MLO, although successful culture and transmission studies are needed for final proof.

Large, bearing coconut palms are difficult to use as test plants in LY transmission experiments. Therefore, immature coconut palms and smaller palm species have been used in most transmission trials. Since symptom expression alone is insufficient for LY identification in these palms, test plants with disease symptoms have routinely undergone electron microscopic examination to determine MLO presence. Because no simpler diagnostic methods exist for LY verification, these electron microscopic studies have been vital to the LY research effort, but electron microscopy is not without its limitations.

Due to the low concentration or absence of MLO in mature parts of a LY-diseased palm, young leaf bases surrounding the apical meristem are collected because these tissues normally contain detectable quantities of MLO. Palms are killed by this collection procedure because they contain only one apical meristem which is destroyed when samples are taken. Thus, an individual palm may be sampled only once.

As evidenced by this study, the success of finding MLO in diseased palms by electron microscopy is not 100%, even in mature coconut palms showing classic LY symptoms. Younger tissues contain more MLO than mature tissues, but often there are too few MLO to detect, even in these tissues. The unpredictable localization of MLO within the sampled tissue also affected the success rate in this study. Sample choice for electron microscopy involves some random selection, and since only a small amount of material is processed for examination, it is quite possible to choose tissues that contain insufficient MLO for detection.

To illustrate the extremely small sample size that can be effectively studied electron microscopically, an estimate can be made of the time required to examine all sections within a small wooden match stick (2 mm x 2 mm x 4 cm). A section for electron microscopy is normally no larger than 1 mm^2 in surface area (ca. 1/4 the cross section of the match stick) and no thicker than 100 nm (ca.

1/250,000 inch). The match stick would contain ca. 1,600,000 sections of this size, and optimistically one section might be cut and then examined with an electron microscope every 20 minutes. At this rate it would take one person, working day and night, over 58 years to give a cursory examination to all the sections from that single match.

Considering the low concentration and unpredictable localization of MLO within palms affected by LY, as well as the limitations of electron microscopy, testing for LY infection is presently an arduous procedure. However, even with its limitations, electron microscopy will continue to be indispensable for LY diagnosis until simpler techniques are developed.

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ATTRACTIVENESS OF DATE AND COCONUT PALMS TO MYNDUS CRUDUS AND OTHER HOMOPTERANS[1]

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Abstract. **Leafhoppers and planthoppers (Homoptera: suborder Auchenorrhyncha) were sampled by sticky traps placed in mature coconut palms,** Cocos nucifera **L., and date palms,** Phoenix **spp., (Mostly** P. canariensis **Hort ex Chab.) in street plantings in southeastern Florida, and by making counts of insects resting on young coconut and young date palms,** P. dactylifera **L. in an experimental planting in Fort Lauderdale.** Myndus crudus **Van Duzee (Cixiidae) was relatively more abundant in traps in mature coconut and date palms in street plantings compared with other Auchenorrhyncha. No statistically significant difference was found between the numbers of** M. crudus **attracted to these 2 palm species.** Idioderma virescens **Van Duzee (Membracidae) was second in abundance, followed by unidentified species of Cicadellidae. When true date palms of 4 varieties ('Deglet Noor', 'Thoory', 'Halawy', 'Zahidi') were sampled by occular examination on 6 different days during warmer months, auchenorrhychous insects were nearly absent, while an average of 22.5-38.1** M. crudus **were counted on young 'Jamaica tall' and 3 different color forms of 'Malayan Dwarf' coconut palms planted in the same grove with the dates.** Cedusa inflata **Ball was also on the coconut palms. The results of sampling from mature trees strengthens the hypothesis that** M. crudus **may be a vector of lethal decline of date palms. The reason for the near absence of** M. crudus **and other Auchenorrhyncha from young true date palms is not known. The testing of varieties in field plantings for resistance to lethal declines may be prolonged because vectors may not feed on young date palms frequently enough to transmit disease.**

In Haiti more than 30 years ago, date palms, *Phoenix dactylifera* L., were affected by a disease that was suspected to be co-identical with lethal yellowing (LY) of coconut palms, *Cocos nucifera* L. (5). The evidence for the co-identity of these 2 diseases was the similarity of the symptoms and the proximity of the affected date palms to LY-infected coconut palms. In the LY affected areas of Florida, lethal declines that are apparently co-identical with LY affect 4 species of date palms, viz., Canary Island date palm, *Phoenix canariensis* Hort ex Chab.; true date palm, *P. dactylifera*; Senegal date palm, *P. reclinata* Jacq.; and India date palm, *P. sylvestris* (L.) Jacq. (9).

Recently, an apparently co-identical disease affecting Canary Island and true date palms was discovered in the Rio Grande Valley of Texas (7). An association with mycoplasmalike organisms (MLO) has been shown for LY and for the date palm lethal declines in Florida and Texas, and for many other palm species in Florida (7, 9). In this report, the term LY will refer to coconut LY and the MLO-associated lethal declines of other palm species.

LY has been known since the 1870's or earlier (6, 8), but prior to 1980 had not been reported outside of coconut growing areas. In LY-affected areas of the Caribbean and Africa, LY of palms other than coconut has not been reported or has received scant attention. In Florida, coconut was apparently the first species affected or observed, after which the disease apparently spread to or was seen in other palms (2). Thus, prior to the recent outbreak of LY in Texas, LY research has concentrated on the disease in coconuts.

The Texas situation raises the possibility that LY could extend into northern Florida and other Gulf Coast states where ornamental date palms are grown in the absence of coconut palms. Also, ornamental palms and commercial

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Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the University of Florida, and does not imply its approval to the exclusion of other products that may be suitable. James DeFilippis assisted in field work and provided the photograph for Fig. 1.