Effects of Lethal Yellowing on the Composition of the Phloem Sap from Coconut Palms in Jamaica

W. P. C. Stemmer, D. B. Archer, M. J. Daniels, A. M. C. Davies, and S. J. Eden-Green

Visiting graduate student, postdoctoral fellow, and principal research scientist, respectively, John Innes Institute, Norwich, England. Fourth author: research scientist, Agricultural Research Council, Food Research Institute, Norwich, England. Fifth author: plant pathologist/entomologist, Overseas Development Administration, Lethal Yellowing Research Team, Coconut Industry Board, Jamaica. Present address of senior author: Department of Plant Pathology, University of Wisconsin, Madison 53706.

The authors wish to express their thanks to Professor J. Van Die, J. Best, J. Longland, M. Gee, J. Eagles, and A. Wong for their contributions.

This research was supported in part by the U.K. Overseas Development Administration.

Accepted for publication 9 July 1981.

ABSTRACT

Stemmer, W. P. C., Archer, D. B., Daniels, M. J., Davies, A. M. C., and Eden-Green, S. J. 1982. Effects of lethal yellowing on the composition of the phloem sap from coconut palms in Jamaica. Phytopathology 72:672-675.

The chemical composition of the phloem sap from healthy and lethal yellowing-diseased coconut palms (*Cocos nucifera*) was analyzed to study the natural habitat of the mycoplasmalike organism found in the sieve tubes of diseased coconut palms. Saps from healthy or diseased palms did not differ significantly in total solids, content; sugar, amino acid, and elemental metal compositions; and protein band patterns on polyacrylamide gels. Phloem sap from their inflorescences, however, differed from trunk sap in total solids content, and in sugar, amino acid, and elemental metal compositions.

Lethal yellowing disease causes extensive destruction of coconut palms (*Cocos nucifera* L.) and other palm species in Florida, Jamaica, and the northern Caribbean. The disease in the Caribbean region is probably either closely related to, or identical with, other lethal diseases of coconut in West Africa and may have originated in South East Asia (7).

The symptoms in foliage, inflorescence, fruits, and roots of coconut palms affected by lethal yellowing have been well documented (5,13,21,24). However, the causal agent of lethal yellowing has not been isolated and there are no methods for early detection of the disease. The association of organisms resembling mycoplasmas with diseased plants has been demonstrated by electron microscopy, but the organisms (1,24,30) have not been cultured successfully. Remission of symptoms after tetracycline therapy also indicates that the disease is caused by a prokaryote (19,22). Acholeplasmas have been isolated from lethal yellowing-diseased coconut palms, but not from healthy ones (13). The authors concluded, however, that the acholeplasmas present in the exudate (which is called toddy) and in the hearts of diseased palms are not the causal agents of lethal yellowing, but rather are co-infecting pathogens or saprophytes. Moreover, there were no signs of transmission to maize plants by leafhoppers (*Dalbulus maidis*) that had been inoculated by microinjection, although the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/06067204/$03.00/0
©1982 The American Phytopathological Society

acholeplasmas survived and multiplied in the insects.

Gross morphological symptoms permit identification of lethal yellowing-infected palms only at relatively advanced stages. An early indicator of the presence of lethal yellowing has been sought in the chemical composition (percent ash, Ca, K, Mg, Na, B, and Mn) of roots and leaves from healthy and diseased palms (17). No clearly defined changes during the course of disease were observed; the range of concentrations for most elements was very wide. Dabek (8) used chemical tests to detect lethal yellowing in Jamaica. After the appearance of lethal yellowing symptoms a significantly lower catalase activity was detected in leaf samples of diseased palms, compared with healthy palms. None of the tests, however, could detect lethal yellowing before the visible symptoms appeared. More recent observations have indicated that measurement of xylem tensions might be used as an indicator of palms in presymptomatic stages of disease (23).

The membrane-bound bodies, resembling mycoplasmas, found in lethal yellowing-infected palms are phloem restricted (1,25,30), so changes in phloem sap may occur early in the disease. In this study we have chemically analyzed the toddy, believed to be phloem sap (28), from many healthy and lethal yellowing-infected palms.

MATERIALS AND METHODS

A total of 31 samples from 25 coconut palms were analyzed, 14 from healthy palms and 17 from palms affected to different degrees by lethal yellowing. Except for two samples of healthy inflorescence sap from cultivars Rangiroa Dwarf and Malayan Dwarf, all samples were obtained from the stems of coconut palms of cultivar Jamaica Tall in Jamaica, as described in a separate paper (15).

Sugar analysis. Analysis of the free sugars in the sap was performed by gas chromatography and mass spectrometry. Freeze-dried sap (10 mg) was derivatized directly by reaction with 100 μl each of hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) (both from Pierce Chemical Co., Rockford, IL 61105). Ribose (0.2 mg) was used as an internal standard. After the addition of 500 μl of water-free pyridine, the samples were heated at 50 C for 30 min, and 1 ml water-free pyridine was then added (2). Samples (4 μl) were injected into a 4-m glass column (2 mm ID) with 3% SE-30 on Diatomite (80- to 100-mesh), run on a Pye-Unicam 104 gas chromatograph with temperature programmed from 155 to 320 C at 2 C/min. Peaks were identified by gas chromatography/mass spectrometry carried out on a 1.5-m glass column (2 mm ID) with 3% OV-1 on Gas Chrom Q (80- to 100-mesh) run on a Sigma-2 gas chromatograph (Perkin-Elmer) coupled by a glass jet separator to an MS 3076 mass spectrometer (Kratos Ltd.) at a resolution of 1,000, an ionizing energy of 70 eV, and a source temperature of 150 C. Spectra were recorded at 10 sec per decade into a DS 50 SM data system (Kratos Ltd.).

Protein estimation. The freeze-dried sap was dissolved in distilled water up to the original concentration. The protein content of aliquots of these sap samples was estimated by a dye-binding assay (3), which is suitable for estimation of protein content in plant extracts (26). Bovine serum albumin (Sigma) was used as the protein standard.

Protein separation on polyacrylamide gels. Sap samples were dialyzed against two changes of distilled water and macromolecules were precipitated with trichloroacetic acid (TCA; final concentration 5%, w/v) at 4 C for 1 hr. Precipitates were recovered by centrifugation at 12,000 *g* for 15 min, washed once in 5% (w/v) TCA, then once in TCA/ethanol (1:1, by vol.) and finally in ethanol. After drying, precipitates were dissolved in 1% (w/v) sodium dodecyl sulphate (SDS), 1 mM ethylenediaminetetraacetic acid (EDTA), 5% (v/v) β-mercaptoethanol, 10% (w/v) glycerol, 30 mM tris-HCl (pH 6.8) at 100 C for 1 min. Proteins were separated in 12% (w/v) acrylamide slab gels containing 0.1% (w/v) SDS (20). Protein bands were stained according to Fairbanks et al (16).

Element analysis. The concentrations of K, Na, Mg, Ca, Fe, Cu, and Zn in the freeze-dried sap samples were determined by atomic absorption spectrometry. One-gram samples of freeze-dried toddy were slowly heated in Pyrex beakers on a hot plate for 2 hr. When the samples appeared to be completely black, they were transferred to a furnace and were ashed at 450 C for 24 hr. After ashing, 10 ml of 6M HCl was added to each sample and the mixture was heated to approximately 80 C for 10 min. Residual carbon particles were filtered out with Whatman No. 1 filter paper disks. After dilution, the concentrations of elements in the filtrates were determined with a Varian Techtron AA 6 atomic absorption spectrophotometer. Two milligrams of K per milliliter was added to the dilutions to suppress ionization of Na and Ca. K, Na, Mg, Zn, Cu, and Fe were determined with an air-acetylene flame; for Ca, a nitrous oxide-acetylene flame was used.

Amino acid analysis. Approximately 30 mg of freeze-dried toddy was dissolved in 2 ml of sodium citrate buffer, pH 1.5 (containing norleucine and aminoguiadinopropionic acid, 0.5 μmol/ml, as internal standards). Aliquots of this solution (50 μl) were analyzed on a Technicon TSM amino acid analyzer (Technicon, Elmhurst, IL 60126) equipped with a microcomputer-controlled gradient elution device (11). The chromatograms were integrated and quantified as described previously (27). The gradient used did not resolve asparagine and glutamine, which may have been quantified as threonine and serine, respectively.

Phytotoxic substances. Lyophilized toddy was dissolved in water at a concentration of 25 mg/ml and extracted twice with an equal volume of water-saturated 1-butanol. The pooled butanol extracts were evaporated to dryness under reduced pressure at 45 C and the solids were dissolved in the original volume of water. A range of dilutions in water was prepared and 0.2-ml portions of each dilution were placed in wells of large microtiter plates. Pieces (2 cm × 1 cm) of Whatman No. 1 filter paper were folded to form paper bridges and inserted in the wells and six lettuce seeds (*Lactuca sativa* 'Winter Density') were placed on the paper. The seeds were free of the liquid, but in contact with the saturated paper. The plates were sealed with transparent tape and left at room temperature for 3–5 days. The dilution of the extract that inhibited seed germination was noted.

Sterol analysis. Freeze-dried sap (10 g) was extracted twice in 100 ml of redistilled chloroform for 2 hr at 20 C. The extracts were pooled, the volume was decreased by evaporation under reduced pressure, and then they were washed (18). The lipid extract was then dried in vacuo and weighed. Saponification of the lipid extract was performed in 3 M methanolic KOH at 80 C for 2 hr. Nonpolar, saponified lipids were then extracted in petroleum ether.

GC/MS was carried out on a 1.5-m glass column (2 mm ID) with 3% SP-2100 on Supelcoport (100- to 120-mesh; Supelco, Inc.) run on a Pye-104 gas chromatograph (Pye-Unicam) coupled by a glass jet separator to a MS 902 mass spectrometer (Kratos Ltd.) at a resolution of 1,000, an ionizing energy of 70 eV, and a source temperature of 200 C. Spectra were recorded at 10 sec per decade into a DS 50 SM data system (Kratos Ltd.).

RESULTS AND DISCUSSION

Although palm toddy samples are believed to consist almost entirely of phloem sap (28,29), a small but varying amount of xylem sap may have been present in the sap samples used. Owing to its lower solute concentration, this xylem sap would hardly affect the composition of the phloem sap solids. For this reason, all data are presented as milligrams per gram of solids and not as milligrams per gram of sap.

Total solids. The concentrations of solids in the sap samples were determined by freeze-drying. With increasing disease severity, total solids in the sap appeared to increase (Table 1). Inflorescence sap had a much higher concentration of solids than sap collected from the trunk of the coconut palm.

Sugar composition. Nine different free sugars, including reducing sugars, were detected in the sap (Table 2). The main sugar was sucrose. A few GC-peaks, each corresponding to less than 0.2% of the dry weight, could not be identified. Two different inositols were present in the sap (inositols A and B). Mass spectrometry showed that they were different inositols, but they could not be characterized further.

No significant differences were observed in the free-sugar concentrations in the sap sampled from the trunk of healthy and diseased palms. Inflorescence sap from healthy palms, however, was found to be different in composition from trunk sap, the most striking difference being the much lower concentrations of both inositols. Unfortunately, it was not possible to obtain sap from inflorescences of Jamaica Tall palms (15) which is the only widely grown cultivar significantly affected by lethal yellowing in Jamaica.

Protein content. Total protein content of the trunk sap from healthy palms was 1.3 mg protein per gram of sap solids. We were not able to detect any clear differences in protein bands in sap from healthy and diseased plants by electrophoretic separation. Antigenic differences have been reported (6) in phloem exudates of healthy and lethal yellowing-diseased palms, but this has not been correlated with a difference in a specific component.

Element analysis. No significant differences were found in the elemental composition of trunk sap from healthy and LY-diseased coconut palms (Table 3). Sap from the inflorescence of healthy palms had an eightfold lower concentration of K, a fourfold lower concentration of Mg, and values for Ca, Zn, and Cu also were lower than in healthy trunk sap (micrograms of the element per gram of solids). Values for Na are high compared to other palms (28,29). One possible explanation for this high Na concentration is the Na-rich natural littoral habitat of coconut palms.

Amino acid composition. The main amino acids in the trunk sap from healthy and LY-diseased coconut palms are glutamic acid, threonine, proline, serine, aspartic acid, and alanine (Table 4). There were no significant differences in the quantities of individual amino acids between healthy and diseased coconut palms. However, there are indications that the relative amounts of some of the amino acids were related to the health of the palm and this aspect is under further investigation. In the inflorescence sap from healthy palms, the main amino acids are glutamic acid, aspartic acid, arginine, threonine, and serine.

During the 44-hr exudation period from trunks of healthy palms, the total concentration of amino acids in the freeze-dried sap decreased steadily to about 80% of the initial value (Table 5). During this exudation period, the concentrations of histidine and arginine in the freeze-dried sap dropped rapidly. Methionine and threonine concentrations dropped slowly. Isoleucine, leucine, serine, and lysine concentrations dropped for 24 hr after the start of sap collection, but then increased to the initial concentrations.

TABLE 1. Total solids in phloem sap of healthy and lethal yellows (LY)-diseased coconut palms

Sample	Samples (no.)	Solids (mg/ml)[a]
Trunk, healthy	6	62 ± 4
Trunk, diseased	12	86 ± 24
LY-stage 2[b]	5	78 ± 14
LY-stages 3–6[b]	7	95 ± 32
Inflorescence, healthy	2	153 ± 28

[a]Average value ± one standard deviation.
[b]Disease severity scales from (14).

TABLE 2. Sugar composition of phloem sap[a] of healthy and lethal yellows (LY)-diseased coconut palms

Compound	Trunk diseased[b] (mg/g)	Trunk healthy[c] (mg/g)	Inflorescence healthy[d] (mg/g)
Sucrose	592 ± 58	561 ± 57	599 ± 25
Inositol A	82 ± 14	101 ± 21	2.5 ± 0.5
Glucose	23 ± 30	29 ± 37	71 ± 36
Fructose	18 ± 22	12 ± 15	59 ± 24
Inositol B	13 ± 3	22 ± 10	0.5 ± 0.0
Mannitol	5.1 ± 8.2	8.1 ± 7.1	5.0 ± 0.9
Oxysuccinate	4.1 ± 3.9	5.2 ± 4.8	...
Deoxyglucose	3.0 ± 1.2	1.7 ± 1.3	0.5 ± 0.1
Raffinose	1.5 ± 3.1	2.7 ± 3.7	6.3 ± 5.4
Totals	742 ± 11	744 ± 13	756 ± 12

[a]Average value ± one standard deviation.
[b]Number of samples, 17.
[c]Number of samples, 6.
[d]Number of samples, 2.
[e]Not detected.

TABLE 3. Elemental chemical composition of phloem sap[a] of healthy and lethal yellows (LY)-diseased coconut palms

Compound	Trunk diseased[b] (μg/g)	Trunk healthy[c] (μg/g)	Inflorescence healthy[d] (μg/g)
K	35,900 ± 8,800	45,300 ± 6,500	4,850 ± 30
Na	1,740 ± 620	2,210 ± 800	2,300 ± 60
Mg	970 ± 200	970 ± 200	240 ± 20
Ca	220 ± 130	140 ± 80	36 ± 6
Zn	29 ± 5	27 ± 9	5.8 ± 1.8
Cu	19 ± 11	15 ± 7	2.7 ± 0.3
Fe	12 ± 9	15 ± 14	11 ± 1

[a]Average value ± one standard deviation.
[b]Number of samples, 17.
[c]Number of samples, 6.
[d]Number of samples, 2.

TABLE 4. Amino acid composition of phloem sap[a] of healthy and lethal yellows (LY)-diseased coconut palms

Compound	Trunk diseased[b] (mg/g)	Trunk healthy[c] (mg/g)	Inflorescence healthy[d] (mg/g)
Aspartic acid	4.33 ± 1.44	5.87 ± 4.17	0.41 ± 0.10
Asparagine/Threonine	11.72 ± 5.27	9.05 ± 6.06	0.33 ± 0.16
Serine/Glutamine	4.80 ± 0.81	4.17 ± 1.37	0.29 ± 0.06
Glutamic acid	11.21 ± 2.24	10.10 ± 1.52	1.10 ± 0.11
Proline	6.33 ± 1.41	5.46 ± 1.50	...[e]
Glycine	0.24 ± 0.06	0.32 ± 0.10	0.05 ± 0.01
Alanine	3.18 ± 1.93	4.21 ± 2.03	0.07 ± 0.01
Valine	1.78 ± 0.64	1.11 ± 0.52	0.02 ± 0.03
Methioine	0.29 ± 0.11	0.17 ± 0.07	
Isoleucine	1.24 ± 0.67	0.62 ± 0.62	0.01 ± 0.01
Leucine	0.48 ± 0.15	0.33 ± 0.18	...[e]
Tyrosine	0.35 ± 0.13	0.29 ± 0.15	...[e]
Phenylalanine	1.14 ± 0.39	1.49 ± 0.42	0.02 ± 0.02
Lysine	0.42 ± 0.19	0.29 ± 0.12	0.02 ± 0.03
Histidine	0.94 ± 0.54	0.34 ± 0.25	...[e]
Ammonia	0.98 ± 0.42	0.70 ± 0.49	0.31 ± 0.03
Arginine	0.54 ± 0.30	0.33 ± 0.13	0.41 ± 0.21
Totals	49.97 ± 0.68	44.85 ± 9.05	3.05 ± 0.59

[a]Average ± standard deviation. Values in milligrams of amino acid per gram freeze-dried phloem sap solids.
[b]Number of samples, 16.
[c]Number of samples, 5.
[d]Number of samples, 2.
[e]Not detected.

TABLE 5. Changes in amino acid composition of trunk phloem sap from healthy palms during exudation

Compound (mg/g)[a]	Time (hr) 0–24	24–36	36–39.5	39.5–44
Histidine	0.37	...[b]	...[b]	...[b]
Arginine	0.92	0.14	0.10	0.13
Methionine	0.31	0.17	0.15	0.14
Threonine	20.57	19.88	13.34	11.93
Isoleucine	0.31	0.10	0.29	0.31
Leucine	0.28	0.04	0.24	0.27
Serine	5.84	...[b]	6.64	5.16
Lysine	0.26	0.04	0.18	0.18
Glutamic acid	10.95	11.62	10.21	11.35
Glycine	0.45	0.46	0.43	0.41
Alanine	5.80	6.48	5.80	6.33

[a]Average values of two palms in milligrams of amino acid per gram of freeze-dried solids.
[b]Not detected.

Glutamic acid, glycine, and alanine concentrations remained unchanged during exudation.

Phytotoxic substances. Butanol extracts of healthy toddy did not usually inhibit seed germination at the highest concentration tested, but about 25% of samples showed inhibitory activity that disappeared when the samples were diluted with one-third volume of water.

We believe that the inhibition was due to inadequate separation of aqueous and organic phases during the extraction. In contrast, extracts from diseased samples were inhibitory at dilutions up to 10-fold, and there was a rough correlation between potency (dilution endpoint) and disease severity. We have not yet attempted to characterize the toxic material. However, the rapid collapse of diseased palms is reminiscent of the behavior of herbaceous plants infected with *Spiroplasma citri* (4), and it has been suggested that a spiroplasma-generated toxin is responsible for this symptom (9).

Sterol composition. Less than 0.01% of the dry sap was extracted by chloroform. *Mycoplasma* and *Spiroplasma* species have an absolute growth requirement for sterol in synthetic media, so it was of interest to analyse the lipid extract for sterol. The nonpolar lipid extract after saponification was subjected to gas chromatography and mass spectrometry. Although many components were separated, some with retention times comparable to authentic sterol standards, amounts were too low for characterization by mass spectrometry.

The mycoplasmalike organism (MLO) associated with lethal yellowing of coconut palms remains uncultured, as do MLOs found in the sieve tubes of other plants. Coconut toddy is available in large amounts, facilitating detailed chemical analysis which, it is hoped, will lead to the rational formulation of synthetic media for the growth of phloem restricted mycoplasmas. A comparison of phloem sap composition with known nutritional requirements of cultivated mycoplasmas has recently been made (10).

The isolation and preliminary characterization of a spiroplasma from coconut palms in Jamaica was recently reported by Eden-Green and Waters (14).

LITERATURE CITED

1. Beakbane, A. B., Slater, C. H. W., and Posnette, A. F. 1972. Mycoplasmas in phloem of coconut, *Cocos nucifera* L., with lethal yellowing disease. J. Hortic. Sci. 47:265.
2. Bhatti T., Chambers, R. E., and Clamp, J. R. 1970. The gas chromatographic properties of biologically important *N*-acetylglucosamine derivatives, monosaccharides, disaccharides, trisaccharides, tetrasaccharides and pentasaccharides. Biochim. Biophys. Acta 222:339-347.
3. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.
4. Calavan, E. C., and Oldfield, G. N. 1979. Symptomatology of spiroplasmal plant diseases. Pages 37–64 in: R. F. Whitcomb and J. G. Tully, eds. The Mycoplasmas. Vol. III. Academic Press, New York.
5. Carter, W., Latta, R. K., and Svah, J. R. R. 1965. The symptoms of lethal yellowing disease of coconut palms. FAO Plant Prot. Bull. 13:49-50.
6. Charudattan, R., and McCoy, R. E. 1975. Antigenic difference in phloem exudates of healthy and lethal yellowing-diseased coconut palms. (Abstr. 226) Proc. Am. Phytopathol. Soc. 2:71.
7. Chiarappa, L. 1979. The probable origin of lethal yellowing and its co-identity with other lethal diseases of coconut. Fifth Session of the FAO Technical Working Party on Coconut Production, Protection and Processing, Manila, Philippines, December 1979.
8. Dabek, A. J. 1974. Biochemistry of coconut palms affected with the lethal yellowing disease in Jamaica. Phytopathol. Z. 81:346-353.
9. Daniels, M. J. 1979. Mechanisms of spiroplasma pathogenicity. Pages 209–277 in: R. F. Whitcomb and J. G. Tully, eds. The Mycoplasmas. Vol. III. Academic Press, New York.
10. Daniels, M. J., Archer, D. B., and Stemmer, W. P. C. 1980. Interaction of wall-free prokaryotes with plants. Sympos. Ser., Soc. Appl. Bacteriol., Academic Press, New York (In press).
11. Davies, A. M. C., Prescott, E. H. A., and Stansfield, R. 1979. Microcomputer-controlled buffer gradient generator for ion-exchange chromatography. J. Chromatog. 171:117-133.
12. Eden-Green, S. J. 1976. Root symptoms in coconut palms affected by lethal yellowing disease in Jamaica. FAO Plant. Prot. Bull. 24:119-122.
13. Eden-Green, S. J., and Tully, J. G. 1979. Isolation of *Acholeplasma* spp. from coconut palms affected by lethal yellowing disease in Jamaica. Curr. Microbiol. 2:311-316.
14. Eden-Green, S. J., and Waters, H. 1981. Isolation and preliminary characterization of a spiroplasma from coconut palms in Jamaica. J. Gen. Microbiol. 124:263-270.
15. Eden-Green, S. J., and Waters, H. 1982. Collection and properties of phloem sap from healthy and lethal yellowing-diseased coconut palms in Jamaica. Phytopathology 72:667-672.
16. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606-2617.
17. Fiskell, J. G. A., Martinez, A. P., and Van Weerdt, L. G. 1959. Chemical studies on the roots and leaves of coconut palms affected by lethal yellowing. Proc. Fla. State Hortic. Soc. 72:408-413.
18. Folch, J., Lees, M., and Sloane-Stanley, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.
19. Hunt, P., Dabek, A. J., and Schuiling, M. 1974. Remission of symptoms following tetracycline treatment of lethal yellowing infected coconut palms. Phytopathol. 64:307-312.
20. Laemmli, U. K., and Favre, M. 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Molec. Biol. 80:575-599.
21. Leach, R. 1946. The unknown disease of coconut palms in Jamaica. Trop. Agric. Trin. 23:50-60.
22. McCoy, R. E., Carroll, V. J., Poucher, C. P., and Gwin, G. H. 1976. Field control of coconut lethal yellowing with oxytetracycline hydrochloride. Phytopathology 66:1148-1150.
23. McDonough, J., and Zimmermann, M. H. 1979. Effect of lethal yellowing on xylem pressure in coconut palms. Principes (Journal of the Palm Society) 23:132-137.
24. Maramorosch, K. 1964. A survey of coconut diseases of unknown etiology. FAO Rome.
25. Plavsic-Banjac, B., Hunt, P., and Maramorosch, K. 1972. Mycoplasmalike bodies associated with lethal yellowing disease of coconut palms. Phytopathology 62:298-299.
26. Robinson, T. 1979. The determination of proteins in plant extracts that contain polyphenols. Plant Sci. Lett. 15:211-216.
27. Stansfield, R., Johnson, M. W., and Couchman, R. 1974. Amino acid analysis—peak integration and identification by computer. Lab. Practice 23:351-355.
28. Van Die, J. 1975. The use of phloem exudates from several representatives of the *Agavaceae* and *Palmae* in the study of translocation of assimilates. Pages 427–466 in: S. Aronoff, J. Dainty, P. R. Gorham, L. M. Srirastava, and C. A. Swanson, eds. Phloem Transport. Plenum Press, New York and London.
29. Van Die, J., and Tammes, P. M. L. 1975. Phloem exudation from monocotyledonous axes. Pages 196–222 in: M. H. Zimmermann and J. A. Milburn, eds. Encyclopedia of Plant Physiology. Vol. I. Springer-Verlag; Berlin, Heidelberg, and New York.
30. Waters, H., and Hunt, P. 1980. The *in vivo* three-dimensional form of a plant mycoplasma-like organism by the analysis of serial ultrathin sections. J. Gen. Microbiol. 116:111-131.