Remission of Symptoms Following Tetracycline Treatment of Lethal Yellowing-Infected Coconut Palms

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ABSTRACT

Of twelve lethal yellowing-infected coconut palms in which a minimum concentration of 3 μg/g tetracycline-HCl was maintained for 5 mo, five showed remission of symptoms, five showed delayed symptom development and two showed no difference, when compared with their controls. The strongest responses occurred in palms which initially had the least leaf yellowing. In treated trees, leaf yellowing and nutfall were not arrested until 10-12 wk after first treatment. Taken in conjunction with the finding of mycoplasmalike organisms in diseased palms, these results strongly support the concept of a mycoplasma etiology for lethal yellowing disease.

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Additional key word: mycoplasma.

Three independent reports have recently associated lethal yellowing disease of coconuts (*Cocos nucifera* L.) in Jamaica with the presence of mycoplasmalike organisms (MLO) in the sieve tubes of leaves and/or inflorescences (1, 7, 15). To date, MLO have been positively identified in material from Jamaica only. Symptomatology, however, suggests that it is the same disease which occurs in W. Africa and in other western Caribbean islands (5, 11) and which recently extended to the Florida mainland. Workers there (16, 17) have claimed mechanical transmission of lethal yellowing to young coconut palms but these results could not be repeated in Jamaica (3, 6). In view of the uncertain nature of the etiology of the disease, infected palms have been treated with tetracycline. Remission of symptoms following such therapy may be taken as additional evidence favoring a mycoplasmal etiology.

The results of tetracycline therapy of other plant diseases suggest that treatment is most effective if applied early. Of the three earliest symptoms of lethal yellowing (nutfall, inflorescence blackening, and leaf yellowing), two are restricted to bearing (older than 7 yr) palms. In the absence of a technique for disease transmission, experiments were thus restricted to naturally infected mature palms.

In preliminary experiments, phytotoxic necrotic flecking was observed in detached leaves which had taken up tetracycline-HCl solutions in excess of 500 μg/ml through their cut petioles and in which the tissue concentration determined by bioassay exceeded 80 μg/ml. Bioassay also revealed that soil drenching and foliar application did not result in tetracycline uptake by palms. A passive trunk injection technique, similar to that used for pear-decline (14) and citrus greening (19), did distribute the antibiotic into the leaves and was therefore adopted.

Sinha and Peterson (20) found an internal oxytetracycline concentration of 50 μg/g, which fell to approximately 10 μg/g within 14 days, to be effective against the clover phyllody agent in aster plants. Klein et al. (10) found appreciably lower concentrations of tetracycline-HCl to be effective against the aster yellows agent. It was found necessary to introduce 20 g tetracycline-HCl into a coconut tree to attain a working concentration of 10 μg/g in the leaves. In the present study the use of 1.0 liter of a 2% solution is a compromise between the limited volume of solution that can be taken up from a trunk borehole and the presumed phytotoxicity at this site of concentrated solutions.

MATERIALS AND METHODS.—Thirty-five approximately 10-yr-old coconut palms of the cultivar 'Jamaica Tall' showing early symptoms of lethal yellowing were selected at Darlingford, Portland, Jamaica. The trees were evaluated for severity of symptoms of nutfall, inflorescence blackening, leaf yellowing, and also for trunk height. On the basis of this evaluation, 12 matching pairs were selected and 11 trees were discarded as unmatchable. Disease symptoms of the chosen pairs ranged from the earliest (blackened open-inflorescence tips, some nutfall, or one or two leaves showing yellowing) to moderately late (severely blackened inflorescences, complete nutfall, and/or up to 15 leaves showing yellowing). On 4 June 1972, one of each pair was randomly chosen for tetracycline treatment. A sloping hole, cut almost completely across the base of the trunk with a 25.4-mm (1-inch) diam auger, was irrigated with 2% aqueous tetracycline-HCl contained in an inverted 1-liter bottle suspended above the hole. The wide-bore tube leading from the bottle was inserted into the hole to a depth which ensured the replenishment of the solution in the hole as it was passively taken up by the transpiration stream. The 12 control trees were similarly irrigated with distilled water. Identical treatments were carried out through freshly bored holes on six later occasions, ending on November 13. The intervals between treatments were gradually extended to a maximum of 5 wk following the results obtained by monitoring the antibiotic level in treated trees by bioassay (see below).

All trees were periodically assessed for disease symptoms. Records were taken 0, 14, 39, 85, 127, 162, 191, and 226 days after the initial treatment. For each palm, records were taken of the number of nuts on each bunch and the condition of leaves and of any newly-opened inflorescences. All leaves up to a position of five above that leaf subtending the presently-open inflorescence were scored as either green, yellow (even if only a few pinnae were affected), or brown (= dead). The few young leaves above this position, crowded in the

center of the palm, are difficult to distinguish by ground observations. The position of each recorded leaf and bunch was fixed by reference to the position of the most-recently opened inflorescence on day-0. By analyzing the records of successive visits, the progress of yellowing and browning of individual leaves and the progress of nutfall from individual bunches could be determined. In addition, the opening of new inflorescences and leaves could be followed.

Tetracycline uptake by trees was monitored by bioassay of leaf extracts, using a technique modified from that of Frederick et al. (4). A fresh sample of 20 g laminar material from approximately 10 leaflets of a symptomless mid-canopy leaf was blended in 100 ml 0.1 M KH_2PO_4 and centrifuged. Three drops of the supernatant extract (or of standard tetracycline solutions) were pipetted into 1-cm diam holes cut in plates of Nutrient Agar (Difco) previously seeded at 50 C with a spore suspension of *Bacillus subtilis*. After incubation overnight at 34 C, the diameter of inhibition of bacterial growth was measured. The concentration of tetracycline in extracts was determined by reference to a standard curve obtained by using pure solutions of tetracycline-HCl. All results were finally expressed as μg tetracycline-HCl/g fresh wt of leaf tissue. The minimum detectable level was 0.4 μg/ml in extracts (= 2.4 μg/g in leaf tissue). Concerning the use of this technique, preliminary experiments had determined: (i) that extracts from untreated healthy or diseased coconut leaves had negligible inhibitory activity against *B. subtilis*; (ii) that the diam of the zone of inhibition by a standard tetracycline solution was unaffected by the presence of coconut leaf extract and; (iii) that following trunk injection of healthy trees with tetracycline, the antibiotic became uniformly distributed in all leaves excepting those which were becoming senescent, reaching a maximum concentration 4-5 days after introduction. Samples of leaves were taken from all treated trees for tetracycline assay (and occasionally, as a check, from

TABLE 1. Initial disease status and results of tetracycline treatment of 12 matching pairs of lethal yellowing-infected coconut palms. Tree-pairs are arranged, as far as variations in the sequence of symptom expression will allow, in order of increasing disease severity at the time treatment started

		Before treatment				After treatment		
Tree[a]	Height of trunk, (m)	Nut-fall[b]	State of open inflor-escence[c]	No. of leaves with symptoms	No. of green leaves	No. of nuts[d]	No. of leaves[e]	Result of treatment
At	4.3	−	−	1	26	17	20	} - Remission
Au	4.6	+	−	1	21	0-127	0-191	
Bt	7.1	−	++	1	26	22	20	} - Remission
Bu	5.9	+	++	1	26	0-127	0-162	
Ct	4.9	+	+	1	25	39	20	} - Remission
Cu	4.0	+	−	10	19	0-127	0-226	
Dt	6.2	+++	−	5	22	19	16	} - Remission
Du	6.5	+++	+	7	28	0-127	0-162	
Et	2.8	+	−	11	17	0-191	0-191	} - Moderate retardation
Eu	3.4	+	+	11	19	0-85	0-162	
Ft	3.7	+++	++	8	19	5	10	} - Temporary remission
Fu	3.4	+++	++	3	15	0-16	0-85	
Gt	4.6	++	++	6	22	2	4	} - Strong retardation
Gu	4.3	++	++	7	26	0-127	0-85	
Ht	3.4	+++	+++	8	17	0-85	0-191	} - Slight retardation
Hu	3.4	+++	+++	10	7	0-39	0-127	
Jt	4.9	++++	+	11	17	6	1	} - Strong retardation
Ju	4.6	+++	+++	9	7	0-39	0-39	
Kt	3.4	++++	+++	7	12	0-0	2	} - Moderate retardation
Ku	4.0	++++	+++	10	6	0-0	0-127	
Lt	6.8	++	−	10	14	0-39	0-85	} - No difference
Lu	7.1	+++	−	8	8	0-85	0-85	
Mt	4.0	++++	+++	11	2	0-0	0-85	} - No difference
Mu	4.6	+++	+++	15	0	0-39	0-0	

[a] A, B, C, etc., identify tree-pairs; t = treated, u = untreated.

[b] Rating scale: − = no nutfall, + = <10%, ++ = 11-50%, +++ = 51-99%, ++++ = 100% nutfall.

[c] Extent of blackening of most recently opened inflorescence: − = none, + = <5%, ++ = 6-50%, +++ = >50%.

[d] Number of nuts remaining at end of observations (226 days after first treatment). An entry 0-85 implies that all nuts had fallen 85 days after treatment started.

[e] Number of green (symptomless) leaves at end of observations (226 days). An entry 0-85 implies that there were no symptomless leaves remaining 85 days after treatment started.

untreated trees also) immediately prior to each tetracycline injection of the trunk.

RESULTS.—For each palm the extent of nutfall, inflorescence blackening, and leaf yellowing at the time of first treatment (day-0) is shown in Table 1. Tree-pairs are arranged in order of increasing disease severity insofar as variations in the sequence of expression of these symptoms allow. Table 1 also shows the final effects of tetracycline treatment. After 226 days, all of the untreated trees were dead or death was imminent; growth had ceased, all nuts had fallen, any remaining leaves were yellow, and the inflorescences were well-blackened. In five of the treated trees (A, B, C, D, F) remission of symptoms was obtained; the upward progression of leaf yellowing and leaf-fall was arrested, opening inflorescences appeared healthy, new nuts were set and new symptomless leaves expanded. Seven mo after the first treatment, the only indications that four of these trees had been diseased were in the reduced number of leaves in the lower part of the canopy and a smaller number of developing nuts than normal. In one tree (F-t) the remission was only temporary; although leaf yellowing and nutfall were arrested by day-85 and new symptomless inflorescences had expanded, the central spear leaves subsequently showed a necrosis and collapse, similar to that occurring in the later stages of the disease in untreated palms, and the latest opening inflorescences were again blackened. Seven mo after first treatment, this palm retained 10 normal green leaves and a few nuts, but had no young leaves expanding from the center. In five other treated trees (E, G, H, J, K), full remission did not occur, but the rates of nutfall and leaf yellowing were

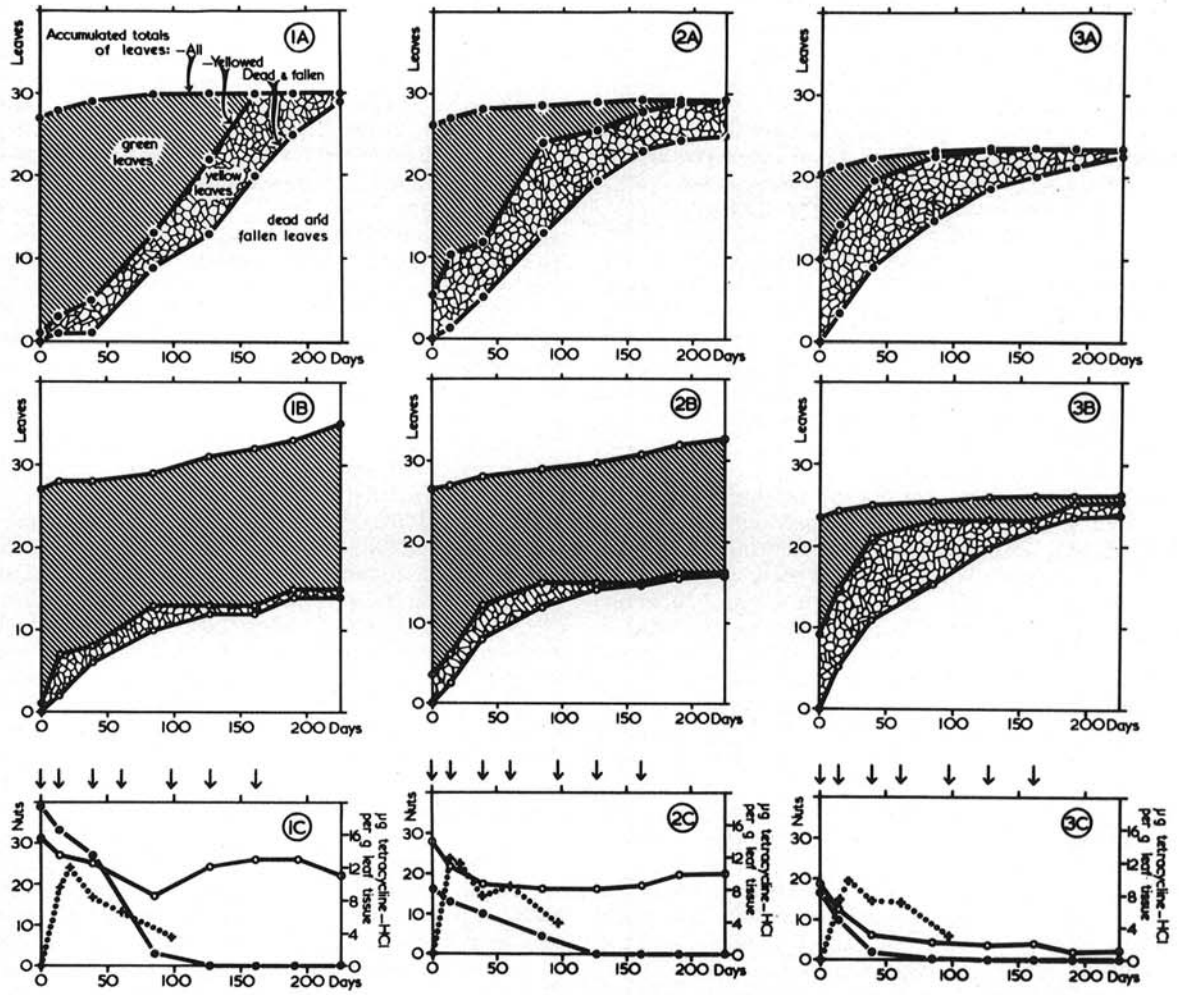

Fig. 1-3. 1-A) Progressive development of leaf symptoms in an untreated lethal yellowing-infected coconut palm. **1-B)** Progressive development of leaf symptoms in a palm, initially matching that in 1-A, showing remission of symptoms following tetracycline therapy. **1-C)** Progress of nutfall from the same two palms (—•—•— untreated; —o—o— treated) and concentration of tetracycline-HCl (µg/g fresh wt) estimated in leaves of treated palm (-x-- x-). Arrows indicate the times of tetracycline application. **2-A to 2-C)** Data similar to that shown in 1-A to 1-C averaged for four other palm-pairs also showing remission. **3-A to 3-C)** Data similar to that shown in 1-A to 1-C averaged for seven palm-pairs which showed retardation of, or no effect upon, symptom development following tetracycline treatment.

retarded compared to their controls. In two cases (L, M), treatment had no observable effect; the trees died at approximately the same time as their controls.

The details of the progress of disease development, together with the records of antibiotic assay are expressed graphically in Fig. 1-3. Figure 1 relates to pair B, representative of those trees which showed remission; the data for the four other trees which exhibited remission (A, C, D, F) were bulked and are expressed as an avg in Fig. 2; for the remaining seven tree-pairs, data are similarly expressed in Fig. 3.

Leaf symptoms.—In the untreated palm B (Fig. 1-A) there were initially 27 leaves of which one, the lowermost, was yellowed. Three new leaves had expanded by day-85, after which time no new leaves or inflorescences opened. Leaf yellowing progressed steadily upward until day-162, by which time all of the original 27 leaves plus the three new leaves were affected. Death of an older leaf follows its first yellowing symptom by approximately 1 mo, but this period is progressively longer as younger leaves become affected. At the end of observations on day-226, only one strongly yellowed leaf remained on this untreated tree. By contrast, in its treated counterpart (Fig. 1-B), new leaves and inflorescences (six in all) continued to expand throughout the observations. Rates of leaf yellowing and death were similar to that of the control over the first 85 days, after which time leaf yellowing ceased abruptly. Subsequently, death of leaves occurred at the same rate as new leaves unfurled, thus maintaining a constant canopy of approximately 20 leaves; this resembles the senescence of a normal healthy palm.

The avg data for the four other tree-pairs showing remission (Fig. 2-A, B) is essentially similar to that already presented for tree-pair B in Fig. 1-A, B. The principal difference lies in the slower rate of leaf yellowing of the four untreated palms but this does not influence the interpretation of the results.

Figure 3-A, B shows that for the remaining seven tree-pairs, ca. 40% of the leaves were already yellowed on day-0. By day-85, the time at which leaf yellowing of remitting trees was arrested (Fig. 1-B, 2-B), very few green leaves remained in this group. However, in the treated palms (Fig. 3-B) the further yellowing and death of these leaves was delayed when compared with the controls (Fig. 3-A). Several of the treated trees in this class showed a residual tuft of green leaves at the top of the canopy of which the petioles broke without yellowing of the pinnae. By day-226 only three of the treated palms retained any green leaves.

In no case was regreening of a yellowed leaf observed.

Nutfall and inflorescence symptoms.—At each recording, the numbers of remaining nuts on individual bunches were counted. Since even in healthy trees there is a self-pruning of newly-set nuts, records for the three youngest open bunches were excluded. Analysis of the results of successive visits showed that nutfall was randomly distributed with respect to the maturity of the other bunches. Therefore, the remaining nuts on all included bunches have been totalled and are presented in Fig. 1-C to 3-C. In the tree-pairs which showed remission (Fig. 1-C, 2-C), nutfall of untreated palms was complete by day-127. By comparison, the rate of nutfall of the treated trees was slowed down after day-39. The subsequent increase in the total number of nuts reflects the cessation of further nutfall from the older bunches and the retention of nuts by the younger bunches. In the remaining seven tree-pairs (Fig. 3-C), nutfall from untreated palms was virtually complete by day-39, whereas in the treated palms a few nuts were retained on some of the trees until the end of observations.

The newly opening inflorescences of untreated palms became progressively more blackened with time; no normal bunches appeared on any of these trees after day-14 excepting palm A-c, where no blackening occurred until day-127. Of the treated palms, two (A, D) never produced blackened inflorescences, eight, which during the early stages showed blackening, subsequently produced normal bunches, and two produced blackened inflorescences throughout.

Bioassay for tetracycline.—Tetracycline-HCl was introduced into treated palms on seven occasions over a 5-mo period (Fig. 1-C to 3-C). To check the uptake and retention of the antibiotic, a leaf sample was removed immediately prior to each of the first five treatments. The concentration of tetracycline estimated by bioassay of the samples thus reflects the minimum levels maintained in leaves throughout the period of treatment. To check the probable maximum levels obtained, an additional sample was taken on day-22, 8 days after the second injection. These data are presented in Fig. 1-C to 3-C. The comparative uniformity of the concentrations maintained over the first 39 days, which includes the one "between treatments" record, suggests that the rates of dispersal and inactivation of tetracycline in palms are slow. On day-22, 8 days after the second treatment, the average concentration was 10.5 μg/g leaf tissue. Seventeen days later, and without further treatment, the concentration had fallen only to 7.7 μg/g. The intervals between subsequent re-treatments were therefore extended to a maximum of 36 days which resulted in a minimum average concentration of 3.5 μg/g.

DISCUSSION.—The maintenance of not less than 3 μg tetracycline-HCl/g fresh wt in leaves of lethal yellowing-infected coconut trees has resulted in remission of symptoms or delayed symptom development in ten of twelve treated trees. It is not yet clear whether the high concentration of tetracycline which has been maintained in the tissues for 5 mo will result in a cure or whether symptoms will recur now that treatment has been stopped. With a latent period in nonbearing palms of 4-5 mo and appreciably longer in bearing palms (8), it will be difficult to distinguish between loss of remission and reinfection should symptoms reappear since the disease is still active at the experiment site.

The response to tetracycline treatment is not immediate. Although effective levels of antibiotic were obtained within 1 wk of first treatment, a comparison of remitting palms and their controls shows that their rates of nutfall and leaf yellowing were similar during the first 39 days and were only fully arrested in remitting trees after 85 days. It would seem therefore that certain irreversible processes are occurring even before symptoms are expressed, which tetracycline is unable to influence. The delayed response to tetracycline offers an explanation why the seven other treated trees did not show full remission. The disease symptoms, especially

those of leaf yellowing, were more advanced at day-0 than those of the remitting trees. By day-85 leaf yellowing and nutfall in two of these trees was complete; thus no response to therapy was possible. On the remaining five treated palms and their controls, a few green leaves and nuts remained. Although these trees have survived as a result of treatment, new growth is minimal, and it seems unlikely that they will recover.

Of the three primary disease symptoms, i.e., nutfall, inflorescence blackening, and leaf yellowing, the latter would seem to be the most important in determining the subsequent response to tetracycline therapy. In the four fully remitted palms, leaf yellowing was slight at the time of first treatment, although nutfall and inflorescence blackening on day-0 varied from severe to nil. Of the treated palms which did not show full remission, all had proportionately more of their leaves yellowed on day-0.

Further experiments are in progress to determine the minimum effective dose of tetracycline and the intervals between re-treatments that may be necessary to maintain a palm in a state of remission. A more refined technique is also required to allow the introduction of larger volumes of a more dilute solution of the antibiotic. A pressure injection, similar to that used by Himelick (9) against Dutch elm disease, would cause less mechanical injury to the trunk and minimize the risk of phytotoxicity at the injection site. With the present cumbersome injection techniques and high costs of antibiotics, commercial chemotherapy seems improbable except to protect certain trees which are valuable as a source of germ plasm. The remission and retardation of symptoms obtained following tetracycline treatment, taken in conjunction with the previous finding of MLO in diseased palms in Jamaica, strongly supports the concept of an MLO etiology for coconut lethal yellowing. Exhaustive tests to implicate bacteria and other microorganisms which might also respond to tetracycline therapy, as the cause of lethal yellowing, have all proved negative (2, 6). In future experiments, other antibiotics will also be tested which will discriminate between the pathogenic mycoplasmas and other disease agents similar to mycoplasmas which have recently been recognized in plants (21).

While the present experiments were in progress, parallel studies were undertaken in Florida. McCoy (12) reported that of ten palms treated with tetracyclines, six showed remission of symptoms in the form of healthy new growth, two showed delayed symptom expression when compared with controls, and two did not respond. With the exception of one of the latter trees, none was exhibiting any leaf yellowing at the time of treatment, diagnosis being made on inflorescence necrosis only. The method of injection was similar to ours but appreciably lower concentrations of antibiotic were used and for most trees only one injection was given; remitting palms received 0.05 - 0.20 g oxytetracycline-HCl or 1.5 g tetracycline-HCl (cf. 20 g tetracycline-HCl on seven occasions in our experiment). In Florida, remission followed treatment within 3 mo to as little as 10 days. The most striking difference between the results is that all of our remitting trees produced about ten yellow leaves during the first 85 days of treatment, whereas in the Florida trees, which received less than 1/100 the quantity of antibiotic, leaf yellowing was completely prevented except in one case. Notwithstanding these differences, taken together with our results, these findings strongly support the previously made assumptions, based largely on symptomatology, that the diseases in Jamaica and Florida are similar or identical. Other plant diseases in which MLO have been implicated are transmissable only by insects or by grafting. The several reports (13, 16, 17, 18) of the mechanical transmission of lethal yellowing in Florida (not reproducible in Jamaica) seem irreconcilable with an MLO etiology. Further evidence that the diseases are similar would be obtained, if MLO could be demonstrated in diseased material from Florida.

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