# Uptake, Translocation, and Persistence of Oxytetracycline in Coconut Palm

Randolph E. McCoy

Assistant Professor, University of Florida, Agricultural Research Center, 3205 S. W. 70th Avenue, Ft. Lauderdale 33314.

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ABSTRACT

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Oxytetracycline-HCl (OTC) residues were measured in coconut palm *(Cocos nucifera)* by microbiological assay of aqueous extracts of freeze-dried or fresh tissue samples. Detectable uptake of OTC was obtained only through the injection of aqueous solutions directly into the trunk of the palms. Soil drenches, foliar sprays, and implantation of solid tablets produced no detectable foliar residues. Residues of OTC as high as 20 μg/g fresh weight were found in foliage within 2 days of trunk injection of 6 g of antibiotic. Most of this material accumulated in the middle and upper fronds of the treated trees. Lower concentrations were found in roots, trunk, unopened spear or bud leaves, older senescing fronds, and in fruit. The half-life of OTC in foliage was about 2 weeks.

*Additional key words:* lethal yellowing, antibiotic assay.

Lethal yellowing (LY), a disease of coconut palm *(Cocos nucifera* L.), has now reached epidemic proportions throughout the West Indies and Florida. Florida has lost an estimated 300,000 coconut palms in seven counties to date, and about 300,000 coconut palms were lost to LY in Jamaica in 1973 alone (4). Mycoplasmalike organisms have been found in association with this disease (2, 8, 17, 18, 22) and tetracycline antibiotics will suppress symptom development and stimulate the production of healthy new growth in LY-affected coconut palms in both Florida (12, 13) and Jamaica (5, 11). In fact, the antibiotic oxytetracycline-HCl (OTC) (Terramycin®, Pfizer, Inc., New York City) has been approved by the United States Environmental Protection Agency as a control measure for LY in Florida (14). The distribution and longevity of OTC injected into coconut palms is therefore of particular interest. This report details the results of residue analyses for oxytetracycline activity in treated coconut palms in Florida and discusses their implications to LY control.

## MATERIALS AND METHODS

**Assay procedure.**—Most procedures for measuring antibiotic concentrations use bioassay tests because they are relatively simple to perform, are highly sensitive, and measure only microbiologically-active material. The tests reported here measured oxytetracycline residues in coconut tissues by using *Bacillus cereus* subsp. *mycoides* (Flugge) Smith et al. as a test organism. Bacto antibiotic assay medium No. 3 (Difco Laboratories, Inc., Detroit, Michigan) plus 1.5% agar adjusted to pH 6.0 with citric acid was used. A suspension of *B. cereus* washed from the surface of agar slants was added to the medium at 40 C before it was poured into plates. Five milliliters of inoculated medium was used per 9-cm diameter petri plate. Standard solutions of OTC ranging from 0.1 to 20.0 μg/ml were made up fresh and a standard curve was prepared for each assay (Fig. 1). Either Lab-Line Bioassay Plates® (Lab-Line, Inc., Melrose Park, Illinois) or paper disks (Bacto Concentration Disks-Sterile Blanks) were used to place the test solutions on the agar surface. Plates were incubated overnight at 30 C and radial zones of bacterial inhibition were read and

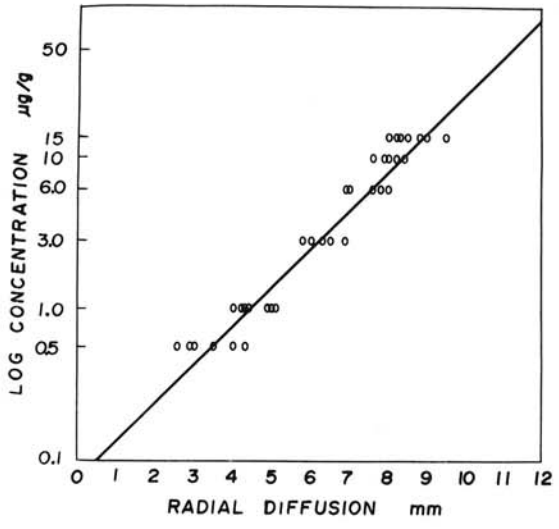


**Fig. 1.** Typical standard curve of oxytetracycline (OTC) concentration versus radial zone of inhibition of growth of *Bacillus cereus* subsp. *mycoides*. Coefficient of linear regression = 0.97.

recorded. Comparison of the test zones with a standard curve gave concentrations of OTC in the test tissue. Ten or 12 readings were made for each test solution evaluated, including standards.

Extracts from leaf, root, or trunk tissues were obtained by freeze-drying 10 g fresh weight samples of pinnae, roots, or sawdust from trunk borings, followed by powdering in a Wiley mill. The samples were reconstituted to 15 g with distilled water, centrifuged at 30,000 *g* for 15 minutes at 10 C, and the clear supernatant solution of about pH 5.5 was decanted and used for the assay. Assays of coconut milk were made directly with no dilutions or adjustments. Assays of coconut meat were made by freezing 10 g fresh weight samples and mashing them by means of a mortar and pestle in 5 ml of 0.1 M phosphate buffer at pH 4.5 to give a final pH of 5.5. Samples from untreated trees or trees prior to treatment were assayed in like manner to determine the lowest

TABLE 1. Mean[a] leaf tissue concentrations of oxytetracycline (OTC) ± standard deviations in μg/g at 3- and 7-day intervals after application of OTC to mature coconut trees

| Injection method | OTC dose per tree: Liters water used | Trees (No.) | 3 days | 7 days |
|---|---|---|---|---|
| Air pressure | 6 g/liter | 5 | 8.0 ± 6.1 | 5.7 ± 3.7 |
| Mauget | 6 g/0.09 | 5 | 15.0 ± 5.0 | 13.8 ± 4.3 |
| Tablet | 6 g/0 | 2 | 0 | 0 |
| Drench | 50 g/65 | 2 | 0 | 0 |
| Spray | 4.3 g/22 | 2 | 1.3 ± 1.3 | 0 |
| Threshold value[b] = 0.5 μg/g | | | Modified control[c] 10 μg/g read 9 μg/g | |

[a]Means are average of 10 readings.
[b]Threshold value for a particular tissue is the day zero or control reading below which concentration cannot be determined.
[c]Modified control values are derived from control tissues to which known amounts of OTC have been added to determine the binding capacity of the tissue for the antibiotic. The second value is the assay reading.

TABLE 2. Oxytetracycline (OTC) concentrations in tissues of three coconut palms air-pressure injected on day zero with 6 g OTC in 1 liter of water. Leaf samples from inner (in) and outer (out) pinnae of each sampled frond. Leaf No. begins with spear and extends down in phyllotaxic order

| Tissue | | Day | Mean[a] OTC concentration μg/g: Tree 1 | Tree 2 | Tree 3 |
|---|---|---|---|---|---|
| Roots | | 7 | 0 | 0.9 | 1.0 |
| Trunk 0.5 m above ground | | 7 | T[b] | 1.2 | 1.6 |
| Trunk 2.0 m above ground | | 7 | T | T | T |
| Milk 1[c] | | 3 | . . . | . . . | 0 |
| Milk 2 | | 7 | . . . | . . . | 0 |
| Milk 3 | | 7 | . . . | . . . | 0 |
| Leaf No. 1 | Spear | 3 | . . . | 1.2 | . . . |
| Leaf No. 5 | in | 3 | 10.5 | 4.2 | 6.3 |
| Leaf No. 5 | out | 3 | 3.0 | 3.5 | 5.1 |
| Leaf No. 11 | in | 3 | 10.1 | 4.4 | 5.6 |
| Leaf No. 11 | out | 3 | 6.0 | 5.9 | 7.0 |
| Leaf No. 17 | in | 3 | 3.1 | 3.9 | 11.4 |
| Leaf No. 17 | out | 3 | 5.7 | 5.6 | 3.2 |
| Leaf No. 23 | in | 3 | 1.1 | 1.2 | 1.9 |
| Leaf No. 23 | out | 3 | 1.5 | 1.5 | 1.3 |
| Leaf No. 1 | Spear | 7 | . . . | 0.8 | . . . |
| Leaf No. 5 | in | 7 | 9.6 | 6.3 | 5.5 |
| Leaf No. 5 | out | 7 | 9.6 | 12.6 | 11.5 |
| Leaf No. 11 | in | 7 | 6.7 | 8.3 | 11.6 |
| Leaf No. 11 | out | 7 | 6.5 | 7.5 | 14.0 |
| Leaf No. 17 | in | 7 | 3.3 | 2.9 | 5.1 |
| Leaf No. 17 | out | 7 | 6.5 | 3.8 | 3.6 |
| Leaf No. 23 | in | 7 | 1.5 | 2.3 | 2.2 |
| Leaf No. 23 | out | 7 | 1.6 | 1.3 | 2.1 |
| THRESHOLD value[d], Leaf 0.4 μg/g | | | MODIFIED CONTROL[e], Leaf 7.5 μg/g 8.4 μg/g | | |
| THRESHOLD value, Roots 0.2 μg/g | | | MODIFIED CONTROL, Root 7.5 μg/g 8.7 μg/g | | |
| THRESHOLD value, Trunk 1.0 μg/g | | | MODIFIED CONTROL, Trunk 7.5 μg/g 6.3 μg/g | | |
| THRESHOLD value, Milk 0.5 μg/ml | | | MODIFIED CONTROL, Milk 10.0 μg/ml 10.5 μg/ml | | |

[a]Each mean is average of 10 readings.
[b]T = Trace (less than threshold value).
[c]Milk samples 1 and 2 from green full-sized fruits, milk sample 3 from green one-third grown fruit. Only tree 3 was bearing fruit.
[d]Threshold value for a particular tissue is the day zero or control reading below which concentration cannot be determined.
[e]Modified control values are derived from control tissues to which known amounts of OTC have been added to determine the binding capacity of the tissue for the antibiotic. The second value is the assay reading.

threshold values at which OTC could be measured in each test. In addition, known concentrations of OTC were added to control samples. These modified control samples were assayed to determine whether the tissue extracts had any binding or inactivating effects on OTC.

**Treatment methods and oxytetracycline formulations.**—Methods for introducing soluble, readily translocatable materials into plant tissues have included sprays, root dips, soil drenches, tissue wicks, and direct injections (3, 7, 11, 15, 19, 21). Methods attempted on coconut palms include foliar sprays, soil drenches, trunk injections with or without pressure, and trunk implantations of solid OTC tablets. The trunk injection methods used included gravity flow, air pressure injection, and the Mauget® Injector (J. J. Mauget Co., Burbank, Calif.) (11, 14, 15). Since palms produce no secondary growth, crown size is fixed within a rather narrow range in mature (bearing) trees and the only size differential is in trunk height. All palms used in this study were mature palms of at least 1 m trunk height.

Two OTC formulations are covered in this report. First is 'Terramycin® Tree Injection Formula' (Pfizer, Inc., New York City) of 20% OTC activity formulated specifically for coconut palm. This formulation contains 20% citric acid as a stabilizer and is bulked with sucrose. The second is 'Agricultural Terramycin®' (Pfizer, Inc.), 17.5% OTC, that was used in the spray and drench treatments.

## RESULTS

**Evaluation of treatment methods, uptake.**—Bioassays of leaf tissues sampled from the upper middle crown of coconut palms were made to determine the efficiency of uptake achieved by the various methods used. Air pressure injections of 6 g active OTC dissolved in 1 liter of water resulted in tissue concentrations after 3 or 7 days in the 3 to 15 μg/g range (Table 1). The highest levels of OTC measured, 20 μg/g, were obtained with the Mauget Injector with concentrated solutions of 6 g OTC in 90 ml water. Six grams of solid OTC pressed into tablets and implanted into holes bored in palm trunks produced no detectable tissue levels of OTC after 3 or 7 days. Soil drenches of 50 g OTC in 65 liters of water per tree, with a wetting agent (Big Sur F239, J&B Associates, Miami Springs, Florida) added to improve soil penetration, resulted in no detectable foliar residues. Also, spraying 22 liters of a 200 μg/ml solution of OTC onto the foliage of several test trees, resulted in only one reading of 2.7 μg/g after 3 days; all other readings were zero.

**Translocation of oxytetracycline within the coconut palm.**—To determine the direction of transport of OTC and the tissues in which it accumulates, each of three mature trees of 3-5 m trunk height was air-pressure injected with 6 g active OTC in 1 liter water. Tissue samples were taken from various portions of the trees prior to treatment on day 0, and 3 and 7 days after injection.

The method of sampling chosen was based upon the phyllotaxy of the trees. Beginning with the folded bud or 'spear' leaf which was numbered one, each frond was numbered in a radial spiral down the trunk proceeding from the youngest to the oldest fronds. Starting with the fifth frond down from the spear, every sixth frond was sampled, thus providing samples from fronds of various ages and from different radial directions around the trunk. In addition, two sets of pinnae were taken from each sampled frond one near the frond base and one near its outer tip.

Oxytetracycline was translocated rapidly into the actively transpiring foliage in the upper mid-crown of the tree (Table 2). Amounts in the 1 μg/g range were present in the spear leaf, older senescing leaves, and in roots and trunk tissues. Only one of the trees was bearing fruit; antibiotic activity was not found in milk from those fruit. The middle and upper fronds had 5-10 μg OTC (per g tissue) equally distributed both in the inner and outer pinnae of each frond as well as radially around the tree. The threshold values and those for modified control samples demonstrate the accuracy and validity of the test. Since values at the threshold level of detecting antibiotic averaged 0.5 μg OTC/g tissue and the values for the modified control were within 1 μg OTC/g tissue of those determined for the standards, antibiotic apparently was not chemically bound to the tissue.

**Oxytetracycline residues in fruit.**—To determine OTC levels in coconut fruit, six additional palms of 5-6 m trunk height were each injected with 6 g active OTC. Fruit from these trees were sampled after 1 week and determinations made of OTC activity in milk and endosperm (meat). Based on data taken from 60 coconuts from noninjected palms, threshold levels were 0.3 μg OTC/g of meat tissue and 0.2 μg OTC/ml of milk; modified control values were within 1 μg OTC/g tissue of the actual concentrations.

Of 482 fruit assayed, only five had detectable levels of OTC in coconut milk, all less than 0.3 μg/ml. Nineteen coconuts had trace amounts of OTC (less than 0.2 μg/ml) in milk. All other coconut milk readings were zero. Of 202 coconuts from which meat samples were assayed, 25 had measurable OTC levels, most of them less than 1.0 μg/g, although one coconut did contain 2.6 μg OTC per gram of meat. An additional 27 coconuts had traces of antimicrobial activity in meat that were less than the threshold level of 0.3 μg/g. One hundred fifty coconuts had no antimicrobial activity in the meat. The overall mean concentration of OTC in coconut meat was 0.11 μg/g.

**Persistence of oxytetracycline in coconut palm.**—The longevity of introduced OTC in coconut tissue could have an important bearing on disease control. The OTC levels were monitored by taking three pinnae samples from the fifth frond below the spear leaf from each of three coconut palms (P1, P2, and P3) that had been pressure-injected with 6 g OTC in 1 liter of water. The mean residue levels present in foliage at various times after injection are shown in Fig. 2. Tree P1 consistently had the lowest levels of antibiotic in the foliage, ranging from about 4 μg/g 3 days after injection to 2 μg/g after 14 days and dropping to 1.1 μg/g 36 days after injection. Tree P2 ranged from 6 μg/g at day 2 to 3 μg/g at day 14, dropping to 1.0 μg/g at days 28 and 36. Tree P3 had levels of 20 μg/g at 2 days, dropping to 10 μg/g at 14 days, and to 5 μg/g by 28 days. Also, a significant decrease in foliar OTC level occurred between days 3 and 7 in all three test palms. The variation in uptake between trees could be related to the fact that tree P3 took up its solution within 1 hour of injection whereas trees P1 and P2 required 8 and 4 hours, respectively, during which time there could have been

some inactivation of the antibiotic within the injection equipment.

## DISCUSSION

These studies indicate that aqueous OTC is readily translocated into the most actively transpiring fronds of trunk-injected coconut palms. The folded spear leaf and the lowest senescing fronds accumulated little antibiotic. This agrees with the preliminary results of Hunt el al. (11) who reported that the antibiotic tetracycline-HCl was evenly distributed in coconut foliage with the exception of senescing fronds. In the present study little OTC activity was detected in the nontranspiring tissues of root, trunk, or fruit, which indicated the xylem was the major pathway of distribution. The uniform radial distribution of OTC throughout the crowns of the treated trees is a result of the interconnecting vascular network in the stems of the Palmae (23). This phenomenon does not normally occur in the stems of dicotyledonous plants that appear to require multiple injection sites spaced radially around the stem to obtain an even distribution of solutes throughout the crown (1, 10, 20).

Oxytetracycline is translocated into the fruit of treated palms at extremely low levels. Trees containing foliar levels up to 20 $\mu$g/g OTC had less than 0.5 $\mu$g/g in the meat of a few coconuts. Seventy-five percent of the coconuts from treated palms had no traces of antimicrobial activity in coconut meat and OTC activity was almost non-existent in coconut milk.

The data in Table 1 show that the method of administration of OTC to coconut palm has an important effect upon the foliar levels obtained. The only consistent method was direct trunk injection of OTC in aqueous solution. Solid implants had little effect, possibly due to the phytotoxicity of high concentrations of OTC in a local area, and through inactivation of the antibiotic during a period of slow solubilization. Spraying has been used with occasional success to apply tetracyclines to plants (3, 19, 21), but spraying produced virtually no uptake in coconut palm. Soil drenches of OTC produced no uptake in coconut palm and it is assumed that the antibiotic was rapidly inactivated in the soil.

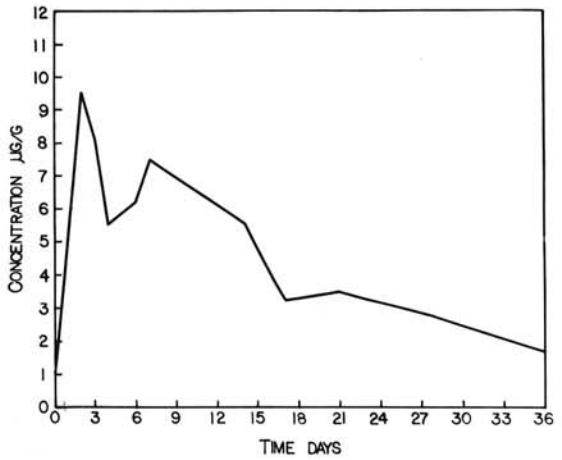


**Fig. 2.** Mean oxytetracycline (OTC) levels in fronds sampled over a 36-day period from the upper middle crown of three mature coconut palms air-pressure injected with 6 g OTC in 1 liter of water.

The maximum level of OTC found in coconut foliage after a 6 g dose was 20 $\mu$g/g, although the mean maximum level was about 6 $\mu$g/g. Since the majority of this activity is found in foliage, the total OTC necessary to produce these levels may be calculated from the total foliage fresh weight of about 200 kg per palm. These figures indicate that only 1.2 g of the 6 g dose must be translocated into the foliage to produce a residue level of 6 $\mu$g/g.

The foliar residues of OTC obtained in this study in relation to dose per tree are three to six times greater than those reported by Hunt et al. (11) for coconut palms treated with tetracycline-HCl in Jamaica. A number of factors may explain these differences, number one among these being that the two antibiotics, while closely related structurally, may be differentially absorbed and translocated within the tree. Another factor that may have reduced the levels reported for the tests in Jamaica was the use of unbuffered tetracycline-HCl in a gravity-feed apparatus that required up to 3 days for absorption. The instability of tetracycline-HCl in aqueous solution is well documented (6). A third factor that may have limited the foliar levels of tetracycline-HCl measured in the studies conducted in Jamaica is the manner of extraction used. It has been virtually impossible to triturate the fibrous pinnae of coconut fronds in a high-speed tissue homogenizer even if the sample was diluted five to six times with water. For this reason fibrous tissues were lyophilized and powdered prior to extraction in this study.

The lyophilization technique allowed the analysis of smaller sample sizes (10 g) which were still sufficiently large to allow a high degree of accuracy. Frederick et al. (7) used only two 12 mm diameter leaf disks per sample in their study on antibiotic uptake in aster. Also, Frederick et al. (7) and Sinha and Peterson (19) did not report specifically whether extracts from untreated control plants exhibited any antimicrobial activity, thereby setting a higher threshold level for the measurement of the antibiotic in tissue than in the standard solutions used for calibration of the test. In addition, modified control values were not provided which would have indicated whether the extracts had any binding action on the antibiotic. I found a low degree of antimicrobial activity in coconut tissues, and thereby raised the threshold for OTC detection in foliage to about 0.5 $\mu$g/g from the absolute threshold of 0.1 to 0.2 $\mu$g/g detected with standard solutions of OTC. Binding of OTC was not observed for modified control extracts of coconut tissues.

The persistence values of OTC in coconut foliage (Fig. 2) indicate a half-life of about 2 weeks. This is somewhat shorter than the 3-week half-life interpolated from the data of Hunt et al. (11) for tetracycline-HCl in coconut. The half-life of oxytetracycline-HCl in aster is about 3-4 days as interpolated from Sinha and Peterson (19). Though OTC may be detected in coconut foliage until about 6 weeks after the administration of a 6 g dose, LY-diseased trees receiving only a 2 g dose, and followed by cessation of symptom development, will produce healthy new growth or remain 'in remission' for 4-7 months (16). The duration of the effect of treatment then, is much longer than the duration of OTC in coconut tissues and,

in fact, is about equivalent to the length of the incubation period of LY as determined by Heinze et al. (9).

It is postulated that the levels of OTC achieved in coconut palm have a toxic effect on the causal agent of LY in that symptom development may be halted for periods 2-5 months longer than the period that OTC may be detected in coconut tissues. The actual levels of OTC that may produce this effect have not yet been determined since the current measurements are from organs, principally leaves, containing several tissue types. The actual effective levels of OTC on a phloem-delimited organism can be determined only from analyses of the phloem contents of treated palms, a subject for a future investigation.

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