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Distribution and Retention of Tetracyclines in Healthy and Likubin-affected Citrus Trees Following Trunk Transfusion*

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ABSTRACT

Solutions of tetracycline-HCl, pH 5.3 (TC), oxytetracycline-HCl, pH 2.6 (OTC), and chlortetracycline-HCl, pH 5.3 (CTC), were transfused by gravity force to the trunk of healthy and Likubin-affected citrus trees or young seedlings. Distribution of the antibiotics and length of their retention in various plant parts were determined by periodic bioassays on plate cultures of *Bacillus cereus* var. *mycoides*. Used at 400 μg/ml or 800 μg/ml, TC was first detected in leaves of healthy trees two days following transfusion. Its activity then increased, reaching a maximum at 6 or 8 days and declined gradually thereafter to become non-detectable at 12-22 days, depending on the trails. Similar observations were obtained with Likubin-affected trees. The highest detectable concentrations in leaves were 10 to 15 μg/ml, when the original solution for transfusion had a concentration of 800 μg/ml. In parallel tests with TC and OTC both at 800 μg/ml, the latter was recovered to a lower level. CTC failed to dissolve at either 800 or 400 μg/ml and its uptake was poor. The antibiotic activity of CTC was barely detectable at 4-6 days following transfusion but was not detectable at other sampling times.

There appears little or no downward translocation of TC from the transfusion point as the antibiotic activity was not detected in the root. Leaves and wood, but not the bark, taken from above the transfusion point contained the activity.

Transfusion with OTC caused the narrowing of citrus leaves developed after the treatment. No similar effect was noted in TC and CTC treated plants.

INTRODUCTION

Citrus is susceptible to the attack by several mycoplasma-like organisms (MLO) with disease symptoms described under various names. The stubborn disease and little leaf of citrus are probably caused by the same organism, *Spiroplasma citri*[27], which can easily be cultured in artificial media[8,20,25] and is capable of multiplication in a wide list of vector[20,23] or non-vector insects[23,32,33]. The organisms associated with citrus greening, decline, leaf mottling and likubin are extensively referred to as mycoplasma-like but they possess a membrane system, the thickness of which has been determined to be 15-25 nm[2,9,25,29,31] as in contrast to the normal thickness of 10 nm for mycoplasmas and spiroplasmas[2,25]. Inspite of these differences, which may be of great significance taxonomically, all the above pathogens found in citrus show a high sensitivity to antibiotics of tetracycline group. Remission of symptoms following tetracycline treatments has been reported for stubborn[13], greening disease[3,26,27], and likubin[5,29,30,31].

The uptake, distribution and retention of

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tetracyclines in citrus have been studied previously in experiments in which young seedlings were used and the antibiotics were introduced mainly through roots[13,31]. However, such a route seems very unlikely to be practical in a field program for disease control. With a few exceptions, studies with other plants along this line have all employed the root absorption route and made use of small plants.

The destructiveness of citrus likubin and the prospect of its control by tetracyclines prompted us to undertake a study in order to gain more information about the distribution and retention of the antibiotics in well-grown citrus plants when test chemicals are introduced by trunk transfusion. This paper describes the results of the study. Parts of experimental data have been published elsewhere[4,5].

MATERIALS AND METHODS

Tetracyclines

Most tests were made with tetracycline-HCl (TC). Either a pure chemical (Achromycin) with 96.7% activity by bioassay or a pre-registration tree injection formulation containing 30.3% tetracycline base was used. Both preparations were a courtesy of the Cyanamid Taiwan Corporation. The test preparation of chlortetracycline-HCl (CTC), with 93% activity (Aureomycin), was also obtained from the Corporation. Both the TC and CTC solutions were brought to pH 5.3 by adding citrate buffer when used. The tree injection TC formulation contained a buffer which gave a pH of 5.3 at the desired concentrations. Oxytetracycline-HCl (OTC) (Terramycin) had a 20% activity and was a tree injection formulation provided by the Pfizer International Inc. It had a pH of 2.6 at working concentrations.

Assay of antibiotic activity

Antibiotic activity of the various tetracyclines was assayed on plate cultures of *Bacillus cereus* subsp. *mycoides* (Flugge) Smith *et al.* in 9 cm diameter petri dishes. The bacteria, ATCC No. 11778, obtained through the Cyanamid Taiwan Corporation, were first cultured on slants of Bacto antibiotic assay medium No. 2 (Difco Laboratories, Inc., Detroit, Michigan), plus 1.5% agar, in a 300 ml flask. Harvest of bacterial spores was made after 6 days at 28°C. The bacterial spores thus harvested were suspended in sterile water and pelleted by centrifugation for 15 min at 2,000 rpm (in an International bench-top centrifuge). After two further washings, they were resuspended in water and adjusted to a concentration of 10^5 cells/ml. One-ml aliquots were stored at 5-10°C for inoculum use.

Each assay dish was filled with 20 ml of the assay agar medium, pH 5.6, (1.5% agar) to which 0.01 ml of a bacterial suspension containing 10^3 cells had been incorporated. Usually 6 filter paper discs (Bacto Concentration-Discs Sterile Blanks), 12.7 mm in diameter, moistened with either the test solutions or with water, were arranged on the agar surface in each dish. Excessive liquid was removed from the discs by touching them with a clean piece of sterile filter paper. The diameter of inhibition zones was measured after an incubation period of 18 hr at 28°C. For each treatment or test concentration 2 discs, except specified otherwise, were used and positioned on the opposite sides in the petridish.

Preparation of samples for bioactivity assay

In activity assays involving leaf tissues from field grown trees, usually 10 mature leaves were sampled from around the canopy 1-1.5 m above the transfusion points. Discs of 0.5 cm in diameter were punched from these leaves and mixed thoroughly. One gram of leaf tissues formed the representative sample and was ground in 10 ml of 0.01 N HCl with a mortar and pestle. The extract was centrifuged for 10 min at 3,000 rpm (in an International bench-top centrifuge) and the supernatant saved to wet the filter paper discs. Samples of wood, bark and roots were similarly processed.

In tests with greenhouse grown young citrus seedlings that had only a limited amount of leaves and other tissues available, a much smaller sample was worked with. Activity was based on a 0.1 g sample for all of plant parts subjected to such tests.

Tree transfusion

For transfusion by gravity[21], solutions of the test tetracyclines were placed in suitable plastic containers holding 1-3 liters, to the bottom of which were attached 1-3 rubber tubings depending on the size of the containers. The

free end of the tubings was connected by means of a hollow metal screw or plastic plug to the hole drilled in the trunk of a citrus tree which was to receive transfusion. Care was taken to remove from the holes as much as possible the sawdusts and the air trapped upon drilling which would otherwise impede transfusion. The uptake varied among trees and were influenced by plant age, vigor and seasons. In the cases of TC and OTC, usually 1 liter of the solutions could be taken up in 17–21 hr by an 8-year old citrus tree through 3 holes on the trunk. The rate of uptake decreased subsequently with time if no new holes were bored and used. Our original intention was to transfuse 1 liter or more to each test tree about 8–10 year old. Actual uptake was usually less, however.

To transfuse 2–3 year old seedlings in the greenhouse, a special injection device was used. The container was a metal cylinder with a side outlet arm near the bottom. Transfusion was also effected by gravity.

Pressure transfusion was performed only in experiments to compare chemical uptake and retention between gravity and pressure transfusions. A stainless steel container of 2 liter capacity was half-filled with test solutions. Air was then pumped in through a valve in a way as a bicycle tire is inflated. A pressure of 2.88 kg/cm² (41.1 lb/in²) was created in this way. The stainless steel container was provided with a single outlet on its bottom to allow the flow of the test solutions into the trees through connections as detailed above for gravity transfusion.

RESULTS

Standard curves of TC, OTC and CTC

Fresh solutions ranging from 0.12–1.96 μg/ml were prepared for TC from the pure chemical (96.7% activity) and for OTC from the tree injection formulation (20% activity). The antibiotics were first dissolved in a small amount of 0.01 N HCl and further brought to desired concentrations in 0.1 M phosphate buffer, pH 4.5. Assay plates, each containing 6 concentration discs, were incubated at 28°C and the radial inhibition zones measured after 18 hr of incubation. The standard concentration curves of both TC and OTC were based on 9 readings for each step in the concentration series. The standard concentration curve of CTC was similarly determined but the fresh dilution series chosen ranged from 0.03–0.48 μg/ml.

The standard concentration curves of TC, OTC and CTC obtained are given in Fig. 1. Obviously, the assay organism, *Bacillus cereus* subsp. *mycoides*, showed a significantly greater sensitivity to CTC than to either TC or OTC. By referring to these standard curves, the antibiotic activity could be measured for samples from various plant parts. However, in actual experiments this was usually done after applying a correction factor. Thus, in each assay a fresh solution at 0.48 μg/ml for both TC and OTC or at 0.12 μg/ml for CTC was always included

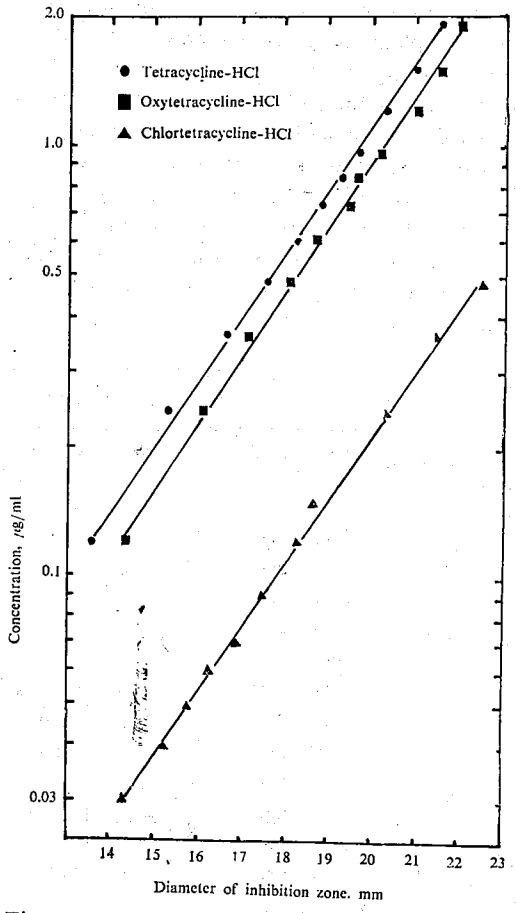


Fig. 1. Standard curves of the concentration of three tetracyclines (TC, OTC and CTC) versus the diameter of inhibition zones on cultures of *Bacillus cereus* subsp. *mycoides*.

and arranged along with the test samples in same assay dish. A comparison of the inhibition zone diameter of the reference disc with that indicated in the standard curve yielded a correction factor for use in the actual calculation of activity of the test samples.

Uptake and retention of different tetracyclines by healthy citrus plant

In four separate tests, 8-year old healthy tankan plants, *Citrus tankan*, were transfused with solutions of different tetracyclines by gravity flow. TC was used at 400 or 800 μg/ml and OTC at 800 μg/ml. The intended concentrations of CTC, 400 μg/ml or 800 μg/ml, were not obtained as this antibiotic failed to dissolve completely under test conditions. Thus, actual concentration was lower than intended.

Bioassays of mature leaf samples taken from test plants at given intervals revealed antibiotic activity at 48 hr but not at 24 hr following transfusion for both TC and OTC. Activity continued to rise with time until a peak was reached at about 6 or 8 days. Then the recovery of activity started to decrease gradually until it fell below the lowest detectable level which was at about 0.12 μg/ml for both antibiotics in our assay. At a test concentration of 800 μg/ml, decline of TC activity below the lowest detectable level occurred at 12 to 20 days, depending on the experiments. At the same test concentration, OTC activity in leaf tissues disappeared 2–4 days earlier than did TC. In general, the uptake and retention patterns of TC and OTC were very similar, except that a slight lower level of activity recovery was obtained with OTC. With CTC which was not completely soluble at intended concentrations, activity was only barely detectable at 4–6 days following transfusion. No antibiotic activity was detected either before or after (Table 1).

Extracts prepared from healthy plants, when assayed parallelly, were not inhibitory to the assay organism.

Uptake and retention of tetracyclines by likubin infected citrus plants

Field grown, 4-year old Ponkan trees, *Citrus poonensis*, with conspicuous likubin symptoms were employed in this experiment. TC and OTC were each transfused at 800 μg/ml to the test trees by gravity. An uptake and retention pattern same as in healthy trees was observed. Highest recovery of activity was obtained at 6–8 days following transfusion and the activity gradually declined thereafter until it fell below the lowest detectable level at 10–16 days for

Table 1. Uptake and retention of different tetracyclines by healthy *Citrus tankan* plants following tree transfusion

Time of experiment	Test compd. & conc. (μg/ml)	Activity recovered at days after transfusion (μg/ml)											
		1	2	4	6	8	10	12	14	16	18	20	22
March 1975	TC 400	0	trace	2.88	5.50	4.10	0	0	—	—	—	—	—
	TC 800	0	3.38	4.80	10.50	4.15	1.78	0	—	—	—	—	—
	CTC 400*	0	0	0	0	0	0	0	—	—	—	—	—
May 1975	TC 800	0	2.45	12.80	15.50	6.30	4.50	1.58	0	—	—	—	—
	OTC 800	0	1.75	4.45	4.20	3.18	2.34	trace	0	—	—	—	—
	CTC 800*	0	0	0.59	0	0	0	0	0	—	—	—	—
July 1975	TC 800	—	3.05	8.60	11.50	6.15	5.45	1.75	trace	0	—	—	—
	OTC 800	—	2.55	5.08	4.60	4.25	4.20	1.45	0	0	—	—	—
	CTC 800*	—	0	0	0	0	0	0	0	0	—	—	—
September 1975	TC 800	—	2.48	10.70	12.05	13.65	10.45	9.08	7.10	3.33	1.77	trace	0
	OTC 800	—	2.90	7.18	8.53	8.05	6.44	3.28	2.05	trace	0	0	0
	CTC 800*	—	0	1.05	0.65	0	0	0	0	0	0	0	0

* Precipitation occurred at this concentration.

The experiment of March 1975 was conducted in TARI's experimental orchard, Taipei and the others in a grower's orchard, Yangmingshan, Taipei. Field grown 8-year-old Tankan trees on Sunki rootstock were used. Three trees were transfused under each treatment. The values given are means of 6 readings on leaf tissues sampled at times as indicated.

Table 2. Uptake and retention of tetracyclines by likubin infected *Citrus poonensis* plants following tree transfusion

Time of experiment	Test compd. & conc. (μg/ml)	Activity recovered at days after transfusion (μg/ml)									
		2	4	6	8	10	12	14	16	18	20
May 1975	TC 800	2.28	7.20	8.30	5.05	3.90	0	—	—	—	—
	OTC 800	1.53	2.10	2.65	2.40	trace	0	—	—	—	—
July 1975	TC 800	3.40	7.98	11.68	10.45	6.85	2.78	0	—	—	—
	OTC 800	2.05	2.80	6.85	4.90	3.70	0	0	—	—	—
September 1975	TC 800	3.84	8.05	10.98	12.27	9.60	8.45	5.20	3.08	2.34	0
	OTC 800	3.50	7.38	8.26	8.30	6.05	2.28	2.20	trace	0	0

The experiments were conducted in Paoshan, Hsinchu on field-grown 4-year old Ponkan trees which were on Rangpur lime rootstock. Three trees were used for each treatment. The values given are means of 6 readings on leaf tissues sampled at times as indicated.

Table 3. Tetracycline-HCl uptake and retention by Valencia orange plants as influenced by method of transfusion

Method of transfusion	Activity recovered at days following transfusion (μg/ml)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
By gravity	0	5.15	—	7.98	—	7.20	—	4.03	—	2.00	—	0	—	0	0
By pressure	9.31	—	10.70	—	10.45	—	9.53	—	8.48	—	7.08	—	4.52	—	0

The experiment employed 8-year old likubin infected Valencia orange plants on Sunki rootstock. For transfusion by gravity force, TC solution was contained in a plastic bottle positioned at about 1 m above the transfusion sites. For pressure transfusion, the container was a stainless steel bottle pressurized to 2.88 kg/cm^2 at the start and 1.68 kg/cm^2 at the end of the transfusion process. A volume of 1 liter at 800 μg/ml was used in either case and the solution was taken up completely in 24 hr. by gravity transfusion and in 5 hr. by pressure. One single plant was used for each treatment.

OTC and 12–20 days for TC (Table 2),

Uptake and retention of TC by gravity vs pressure transfusion

A test to compare gravity flow with pressure transfusion was conducted with 8-year old field grown Valencia orange plants on Sunki rootstock. Antibiotic activity in the leaf tissues was determined at given intervals following transfusion. In the case of pressure transfusion, the uptake of a one liter volume of TC solution, 800 μg/ml, was completed in 5 hr. There was a rapid rise in antibiotic activity in leaves above the transfusion points, a high activity level of 9.31 μg/g being detected in the 24 hr leaf samples. Such activity level was maintained for 8 more days. Leaf samples taken 13 days after transfusion still contained a significant amount of activity (Table 3). By gravity flow, the test plant uptook the same transfusion volume in 24 hr. Leaf samples assayed 1 day after the start of transfusion contained no activity. At 2–10 days during which activity was detected the recovery level was considerably lower than that in pressure transfusion. Evidently, pressure transfusion had the advantages of effecting an improved uptake, a rapid rise and a long retention of antibiotic activity in the test plants.

Tests on the number of transfusions for given dosages

There have been several previous attempts to improve tetracycline uptake by transfusion with concentrates. This, however, often led to phytotoxicity to the xylem tissues around the transfusion points (McCoy, personal communication). We determined the uptake and retention of antibiotic activity, under both the greenhouse and field conditions, for a given TC dosage in single or split applications. In the greenhouse test, a single transfusion of 10 ml TC at 800 μg/ml on day zero was compared with 3 transfusions of same volume but at one third this strength on days 0, 3 and 6 to 2-year

old Rangpur lime seedlings. There was a slower rise in activity in the leaf tissues with the 3-transfusion scheme than with one transfusion, although a detectable level of activity lasted longer in the former case (Table 4). In the field experiment with Valencia oranges, each tree received 600 mg TC at 1,200 μg/ml for a single transfusion treatment and at one third this strength for the 3-transfusion treatment. The differences in uptake and retention patterns with treatments, as described for Rangpur lime, were again observed (Table 4).

Distribution of TC activity in plant parts

The distribution of TC activity in plants was determined in an experiment employing two 2-year-old pot-grown Rangpur lime seedlings. To each seedling a 10 ml volume of TC solution at 800 μg/ml was transfused by gravity. Samples from various plant parts were taken and assayed at 2-day intervals.

In Rangpur lime seedlings, TC activity moved upward from the transfusion point and was detected in the leaves and wood, but not in the bark. There was no downward movement, as recovery tests could reveal no detectable activity in the rootlets. At 4 days, while the activity remained at the same or at a slightly lower level in the wood, it reached a peak in leaves at all stages of maturity. This high level of activity was maintained for a longer time in young than in mature leaves. By comparison,

Table 4. Uptake and recovery of tetracycline-HCl in citrus given different number of transfusions for same dosages

Expt. No.	Test plant		Total TC rate (mg/tree)	No. transfusion	Activity level at days after first transfusion (μg/ml)							
	Variety	Age (yr.)			2	4	6	8	10	12	14	16
1	Rangpur	2	8	1	1.45	4.15	3.53	2.58	trace	0	0	0
	Rangpur	2	8	3	trace	2.10	2.28	3.75	3.20	2.50	1.40	0
2	Valencia	9	600	1	2.32	7.85	6.98	3.03	trace	0	0	0
	Valencia	9	600	3	0	1.78	3.25	7.04	7.87	8.05	7.64	6.98

In experiment 1, the greenhouse grown seedlings of Rangpur lime were each given 8 mg tetracycline-HCl according to the following two different regiments: 1) One single transfusion at day zero of 10 ml of a 800 μg/ml solution; and 2) three transfusions each of 10 ml at 1/3 of the above strength made at days 0, 3 and 6, respectively. Two seedlings were used for each regiment. In experiment 2, the test Valencia plants were on Sunki rootstock. They each received 600 mg tetracycline-HCl either in one transfusion volume at day zero or in 3 transfusion volumes, respectively, at days 0, 3 and 6 as in experiment 1. Each transfusion volume consisted of 500 ml. Two trees were used for each regiment.

Table 5. Translocation and retention of tetracycline-HCl in plant parts of Rangpur lime seedlings following transfusion

Plant tissue	Activity recovered at days following transfusion (μg/ml)						
	2	4	6	8	10	12	14
Mature leaf	1.45	4.15	3.53	2.58	trace	0	0
Two-thirds maturing leaf	1.75	4.43	3.82	2.55	1.70	0	0
Young leaf	3.05	4.57	4.48	3.33	2.78	trace	0
Wood	3.82	3.75	2.05	1.53	0	0	0
Bark	0	0	0	0	0	0	0
Rootlet	0	0	0	0	0	0	0

The experiment employed two 2-year-old pot-grown Rangpur lime seedlings into each of which a 10 ml volume of tetracycline-HCl at 800 μg/ml was transfused by gravity. At 2-day intervals following transfusion samples were taken from different plant parts as indicated and assayed for antibiotic activity. The sampling area was about 40 cm above the transfusion point for leaves, wood and bark. The root samples were dug up from random sites in the potted soil. A 0.5 g weight of the mixed samples for each plant part was ground and its extract assayed.

the wood appeared to acquire, as well as to lose, activity more quickly than did the leaves (Table 5).

In another experiment using three 3-year old sweet orange seedlings, there was again a lack of downward movement of TC activity as evident from bioassays made on daily root samples up to 9 days after transfusion. TC concentration for this test was 800 μg/ml, and transfusion volume per seedling was 10 ml.

Effect on the width to length ratio of citrus leaves

Transfusion with OTC appeared to cause the narrowing of citrus leaves. A random sample of 100 current season leaves from Ponkan trees receiving OTC transfusion at 400 μg/ml yielded a mean width to length ratio of 0.29 while the comparable values for leaves from check plants were 0.48. No similar effect was observed in TC or CTC treated plants.

DISCUSSION

Antibiotics of tetracycline group have been widely tested as control agent against plant diseases due to infection with MLOs[1,3,6,12,13,14,15,17,19,21,24,26,27,30]. The chemicals are either applied as leaf sprays, soil drenches, root dippings onto unwound plant surface or introduced by way of artificial holes drilled in the trunk of test plants. In the latter case, test solutions are either infused by gravity or injected by pressure. Leaf sprays have been repeatedly shown to be very inefficient as a way of introducing antibiotics[14,16,19,24,28]. Soil drenching generally requires a high dosage and, in the case of oxytetracycline-HCl, much of the antibiotic activity has been found to be immobilized by clay and organic substances in the soil, rendering the activity uptake by plants rather inadequate[10]. By root dipping the antibiotics can be taken up easily by plants, but it has little value as a field operation in disease control. Therefore, tree injection either by gravity or by pressure has become a method of choice for applying antibiotics in actual field programs against plant diseases of MLO etiology[3,16,17,18,19,21,24,26,31].

Of the three tetracycline antibiotics, namely, tetracycline-HCl (Achromycin, TC), oxytetracycline-HCl (Terramycin, OTC), and chlortetracycline-HCl (Aureomycin, CTC) used in our study, satisfactory uptake and retention were observed with both TC and OTC. The uptake of CTC by citrus plants was poor and, when there was some uptake, it persisted only for a short time in the treated plants. Low solubility of CTC under the test pH of 5.3 might have accounted in a large part for the results, though other factors could also be involved. As demonstrated by Igwegbe and Calavan[13], CTC was probably easily modified in plants and no compound could be identified with it chromatographically in leaf extracts from plants receiving root dipping treatment. This was in contrast to TC which was taken up by roots and translocated to the aerial parts in unchanged form.

The patterns of activity recovery from plant parts as we observed with TC and OTC after tree transfusion are, in general, consistent with the results of previous investigators[7,18,22,24,28]. However, there was consistently a longer persistence in the plant tissues with TC activity than with OTC, when the two antibiotics were compared at same concentrations. Whether this could be explained in the difference of test conditions, pH 5.3 for TC solutions and pH 2.6 for OTC solutions, or in the intrinsic properties of the chemicals remains to be determined. Working with peaches, Rosenberger and Jones[24] reported detection of OTC activity in plants several months following tree injection. In the soil, OTC has been shown to remain biologically active at a relatively high level (2.5 μg/ml) for 40 days and at a low level (1 μg/ml) one and a half years following soil drenching application[10]. No similar studies have been reported for TC.

Igwegbe and Calavan[13] have studied the distribution of tetracycline activity in citrus plants following root dipping treatment. Activity was found to appear most rapidly and to occur at the highest level in the root. The activity was comparatively low in stems and leaves. In our study in which antibiotics were transfused to the tree trunk, the major activity was found in leaves of varying developmental stages and in wood above the points of transfusion. Appearance as well as loss of antibiotic activity occurred sooner in wood than in leaves, however. No recovery of antibiotic activity

was obtained from rootlets, a result indicating a lack of downward movement of the antibiotics under our test conditions. Again, there was no detectable activity in the bark above the transfusion points.

Tetracyclines have been shown to remit symptoms of stubborn, greening, leaf mottling and likubin on citrus[13,26,27,30,31]. However, the effect must not be a permanent one, because, in the case of likubin at least, the target organism has been found to be present in both the above- and under-ground parts of diseased plants[11], with phloem being the localization site[2,9]. Those MLO cells living in root tissues and in the bark could easily escape the inhibiting effect of the antibiotics which move in one direction only, e. g., upwardly, and did not seem to reach the bark in our tests.

The level of antibiotic uptake and retention in the test plants did not differ between healthy and likubin-infected plants, but it varied with seasons. Retention of activity was considerably longer with September applications than with applications made in May or July (Tables 1, 2). Such variations might have been related to tree activities in different seasons. Further experiments in this respect are in progress.

The application of antibiotics on bearing plants of citrus inescapably raises the question of chemical residues in mature fruits. However, since the longest retention period of TC activity in leaf tissues, as observed in our experiments, did not exceed 24 days after transfusion, the residue problem should not constitute a cause of concern, if due attention has been given to the timing of chemical application and fruit harvesting. Our unpublished results show a lack of detectable TC activity in both the peel and flesh of citrus fruits picked at two day intervals during the period of 2-24 days, following transfusion with a 1200 μg/ml solution of TC.

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灌注樹幹後四環素在健全及立枯病柑桔樹體之分佈與殘存

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三種四環素抗生素之溶液，卽 Tetracycline-HCl, pH 5.3 (TC), Oxytetracycline-HCl, pH 2.6 (OTC)，及 Chlortetracycline-HCl, pH 5.3 (CTC)，曾於田間及溫室試驗中，用以灌注健全或罹立枯病柑桔樹之樹幹及幼苗。灌注後定期自植株不同部位採取樣品，利用 *Bacillus cereus* var *mycoides* 平板培養對供試抗生素在植株內各部位之分佈及殘存時間，加以測定。TC 藥液濃度爲 400 μg/ml 或 800 μg/ml時，其活性可於灌注健樹後二天在葉部檢出。隨後活性漸增，至第 6 天或第 8 天達於最高峯，自此，活性再逐漸下降，於灌注後之第 12 至 22 天間，——視試驗而異——，便消失不見。立枯病株接受灌注後，抗生素之殘留情形，大致與上述相同。

灌注濃度爲時 800 μg/ml，葉部可檢出的 TC 最高濃度爲 10～15 μg/ml，在比較試驗中，葉部檢出之 TC 濃度常比 OTC 爲高。CTC 在 400 與 800 μg 時，不能完全溶解，吸收情形甚差。僅於灌注後第 4 至第 6 天，可於葉部檢出其活性，其他取樣時間均無法檢出。

TC 灌注後未能自柑桔樹根部檢出其活性，顯示其在柑桔樹體內不能自灌注點向下運行。採自灌注部位上方之葉片與木質，均含活性，但樹皮組織無之。

OTC 灌注於柑桔樹，使新生葉片變爲狹長，TC 及 CTC 則無此影響。