Symptom Remission in X-Diseased Peach Trees as Affected by Date, Method, and Rate of Application of Oxytetracycline-HCl

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ABSTRACT

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X-diseased peach trees with 9- to 17-cm trunk diameters were treated at various times during the growing season with five rates of oxytetracycline-HCl (OTC). Injections of 1.25, 2.5, and 3.75 g OTC per tree in September induced remission of symptoms for one year, whereas spring, summer, or fall injections of 0.5 or 0.9 g OTC per tree were less effective. Injections of 1.25 and 2.5 g OTC per tree in October and November were phytotoxic. Injections of dilute OTC by infusion and by pressure, and concentrated OTC pipetted directly into holes drilled in the trunks, all provided remission of foliar symptoms for one year. Terramycinlike activity (TLA) was greatest in leaves from trees injected by infusion. Injection of concentrated OTC was the most rapid and convenient method tested, but 2.5 g OTC in concentrated form caused some necrosis around the injection holes in the tree trunks. Increasing solution concentration by reducing the volume of solution injected did not reduce TLA activity in leaves except for the most concentrated treatment, 1.25 g OTC injected in 10 ml of solution. Terramycinlike activity in leaves declined rapidly following September injections and TLA in fruit from September-treated trees was below a desired residue tolerance of 0.1 μg/g fruit tissue.

Additional key words: mycoplasma, *Prunus persica*.

Although Stoddard reported suppression of symptoms of X-disease of peach using chemical treatments (20), control currently depends on eradicating infected chokecherry plants (*Prunus virginiana* L.) near commercial orchards (6, 10, 16). In 1967, mycoplasma-like organisms (MLO) were reported in phloem cells of plants affected by several "yellows" diseases (1), and tetracycline antibiotics caused remission of symptoms in one of these diseases (8). Subsequently, MLO's were found in phloem cells of peach trees affected by both X-disease and western X-disease (3, 9, 11, 12), and tetracycline treatments produced remission of symptoms (13, 18).

Methods for experimental applications of tetracycline reviewed by Schwarz (19) include root dips, sprays, infusions, pressure injections, and the application of concentrated pastes. Because tetracycline sprays generally proved to be ineffective (15), various methods of trunk injection have been used to treat diseased trees. In large field trials, tetracycline infusions were effectively used to control pear decline (14). Lethal yellowing of coconut palms has been controlled with similar treatment (7). Tetracyclines currently are used commercially for control of both pear decline and lethal yellowing.

Although X-diseased peach trees respond to tetracycline treatments, optimum chemical rates and treatment dates have not been defined fully and application methods have not been compared. The objectives of this study were to determine: (i) the most practical and effective method for treating X-diseased peach trees, (ii) the amount of chemical necessary to achieve symptom remission for at least one year, and (iii) the best timing for treatment. A preliminary account of these findings has been published (17).

MATERIALS AND METHODS

Two wettable powder formulations of oxytetracycline-HCl (OTC) containing the equivalent of 20% oxytetracycline base were used. The formulation for treating pear decline (EPA Reg. No. 1007-79) was tested in 1973, 1974, and 1975, whereas that for treating lethal yellowing of coconut palms (EPA Reg. No. 1007-80) was tested only in 1975. Rates of OTC are given in grams of active ingredient injected per tree.

In 1973, peach trees (*Prunus persica* Batsch 'Glohaven') with X-disease were sprayed weekly for 5 weeks starting 3 May, about 6 weeks before symptoms usually appear. About 15 liters per tree of OTC solution (100 μg/ml) were applied using a handgun. Other trees were injected with 0.5 g OTC per tree on 10 May, 22 June, or 19 July, or with 0.5, 0.9, 1.25, or 2.5 g OTC per tree in early September.

Trees were 6-10 years old with 9- to 17-cm diameter trunks.

Injections were made by the gravity infusion method of Nyland and Moller (14) into three holes per tree, drilled 4-cm deep with a 7-mm diameter bit, and spaced equally around the trunk, about 30 cm above the ground. Location of scaffold limbs was not considered in hole placement, but we avoided dead, sunken, or flattened areas in the trunk.

During autumn 1974, 16 treatments (Table 1) were applied to X-diseased Red Haven peach trees in a 7-year-old orchard, and in September 1975, six treatments were applied in another Red Haven orchard. Each treatment was replicated on four trees. Trees were selected and data were analyzed in blocked design based on the diameter of the tree trunks 30 cm above the ground.

Application methods in 1974 were gravity infusion, pressure injection, and injection of concentrates. Infusions were applied as described for 1973 treatments. Pressure injections were made at 2.8 kg/cm^2 (40 psi) through three holes in the trunk with a Model 102-C pressure injector from the Elm Research Institute, Harrisville, New Hampshire. The pressure and infusion methods were tested at rates of 1.25, 2.5, and 3.75 g OTC per tree. For concentrated injections (18) of 1.25 and 2.5 g OTC per tree, seven holes 10 mm in diameter were drilled at a downward angle of 45 degrees and in a spiral pattern around the trunk. Several milliliters of a concentrated OTC solution were pipetted into each hole. After uptake of solution, injection sites were sealed with wound dressing amended with benomyl.

Treatment dates were 12 and 13 September, 10 October, and 5 November. The effect of solution concentration was tested in 1974 using pressure injections of 1.25 and 2.5 g OTC per tree in 1.89, 3.79, and 7.58 liters of water and in 1975 using infusion of 1.25 g OTC per tree in final volumes of 10 ml and 0.94 and 3.79 liters. The two formulations of OTC were compared in 1975 using infusions of 1.25 g OTC per tree.

Treatments were compared for phytotoxicity, suppression of symptoms, and terramycinlike activity (TLA) in leaves. For assay of TLA, samples of 25 leaves per tree were collected weekly for 4 weeks after treatment on 12 September 1974. Samples of 50 leaves per tree were collected 23 May and 19 June from all October and November treatments and from September infusion treatments. Samples of 40 leaves per tree were collected 10 and 17 September and 23 October from the 1975 treatments. Leaves were taken at random from the center and the periphery of all trees. Samples were held at −20 C until assayed.

Terramycinlike activity was determined by the agar diffusion method (4) using paper assay disks (2). Weighed leaf samples were blended in phosphate buffer (pH 4.5) and vacuum-filtered through Whatman No. 2 filter paper. Filtrates were adjusted to pH 6.8 with 5 N NaOH. Assay disks impregnated with filtrate were placed on agar seeded with *Bacillus cereus* var. *mycoides*. Each time samples were tested, technical OTC (92.7%) was added to extract from healthy leaves to give standard concentrations of 0.1, 0.16, 0.32, 0.63, 1.25, 2.5, 5.0, and 10.0 μg OTC per milliliter of extract. The diameters of

TABLE 1. Oxytetracycline-HCl (OTC) treatments applied to mature X-diseased peach trees in 1974, with results of leaf assays and 1975 ratings for phytotoxicity to foliage

Treatment number	Application method	OTC rate (grams a.i. per tree)	Solution injected (liters)	Treatment date (1974)	Foliage phytoxicity rating[a]	Terramycinlike activity in leaves (μg/g)	
						Autumn[b]	Spring[c]
1	Infusion[d]	1.25	3.79	9/12	1.4	14.74	0.41
2	Infusion	2.50	3.79	9/12	2.4	26.98	0.68
3	Infusion	3.75	3.79	9/12	2.9	35.24	
4	Pressure	1.25	3.79	9/12	1.1	13.21	
5	Pressure	2.50	3.79	9/12	2.1	15.63	
6	Pressure	3.75	3.79	9/12	2.7	26.21	
7	Pressure	1.25	7.58	9/12	1.1	12.12	
8	Pressure	2.50	7.58	9/12	1.8	18.34	
9	Pressure	1.25	1.89	9/12	1.0	13.16	
10	Pressure	2.50	1.89	9/12	1.1	21.24	
11	Concentrate	1.25	10 ml	9/12	1.0	9.63	
12	Concentrate	2.50	17 ml	9/12	1.2	19.33	
13	Infusion	1.25	3.79	10/10	3.7		1.35
14	Infusion	2.50	3.79	10/10	4.8		3.58
15	Infusion	1.25	3.79	11/5	3.2		3.14
16	Infusion	2.50	3.79	11/5	3.9		3.68
17	Control	0	0	. . .	1.4	<0.30	<0.30

[a]Phytotoxicity to foliage was rated 23 May and 15 June 1975: 1.0 = normal foliage development; 2.0 = yellow foliage; 3.0 = yellow foliage with approximately one-half of tree showing stunted foliage development; 4.0 = foliage development severely stunted throughout the tree; and 5.0 = severely stunted foliage with some death of limbs. Ratings are means of four trees and two observation dates.

[b]Autumn leaf samples consisted of 25 leaves per tree collected 19 September, 24 September, 3 October, and 10 October 1974 from each of four replicates.

[c]Spring leaf samples consisted of 50 leaves per tree collected from each of four replicates on 23 May and 19 June 1975.

[d]Pressure injections (2.8 kg/cm^2) and infusions were applied through three holes 7 mm in diameter drilled 4 cm into the trunk 30 cm above ground. For concentrated injections, solution was pipetted directly into seven 10-mm diameter holes drilled 4 cm into the trunks.

inhibition zones produced by the standard concentrations were measured and an equation relating inhibition zone to $\log_{10}$ OTC concentration was derived by linear regression. This equation was used to convert the inhibition zones of sample extracts to micrograms of TLA per milliliter of extract. For samples with activity exceeding 10 μg/g of leaf tissue, extracts were diluted with buffer to allow measurement in the 0.16-10.0 μg/ml range of standard concentrations. The final activity in samples was expressed in micrograms TLA per gram of fresh leaf weight. The minimum detectable level of activity with this technique was 0.16 μg/ml of extract or 0.30 μg/g of leaf tissue. No zones of inhibition were produced by extracts from untreated healthy or diseased trees.

Fruit samples (approximately 2.2 kg) for residue analysis were collected at harvest from most 1973 and 1974 treatments. These samples were held at −20 C until the soluble solids were extracted by homogenizing and straining 100-g subsamples. Clear supernatant solution containing the soluble solids was freeze-dried. Samples later were dissolved in 0.1 M phosphate buffer (pH 6.8) to a final volume of 25 ml, and this solution was analyzed for TLA in the laboratories of Pfizer Inc. by the method of Grove and Randall (4).

The 1974 treatments were rated in 1975 for symptom remission, damage to the tree trunks, and toxicity to foliage. Trees were checked for X-disease symptoms in early September and a tree was considered diseased if symptoms occurred on any branch.

Each tree was examined for possible trunk damage at least twice and those showing severe damage were noted. Control trees were not rated since holes were not drilled in them.

Toxicity to foliage was rated using a scale of 1.0 (= no phytotoxicity) to 5.0 (= severe phytotoxicity). Ratings were made on 23 May and again on 5 June, and the results were averaged.

RESULTS

Trees sprayed with a 100 μg/ml OTC solution during May 1973 developed X-disease symptoms 2 weeks after symptoms appeared on untreated trees. By September, sprayed trees did not differ from controls. Infusion of 0.5 g OTC per tree on 10 May delayed the appearance of leaf symptoms for 4 weeks and decreased the rate of symptom development during the remainder of the season. Infusions in June and July checked further symptom development that season, but none of the 1973 spring or summer treatments affected symptom development in 1974.

Injections of 0.5 g OTC per tree in September 1973 delayed the onset of X-disease symptoms by several weeks in 1974, whereas 0.9 g delayed symptom onset about 7 weeks. Trees injected with 1.25 or 2.5 g OTC per tree in September 1973 developed no X-disease symptoms during 1974. Trees treated during September 1973 were treated again with 1.25 or 2.5 g OTC in September 1974 and remained symptomless through 1975.

All 16 treatments applied in autumn 1974 gave remission of foliar symptoms through 1975, whereas control trees exhibited leaf symptoms in July and extensive defoliation in September. Fruit on treated trees was similar in size to fruit on healthy trees; control trees produced small fruit which usually dropped before ripening.

The time required for infusion of the solution varied with weather conditions and with time of year. In 1973, uptake of 3.79 liters of solution required about 2 weeks in May, 5-7 days in June and July, and 1-3 days in September. September infusions in 1974 and 1975 required up to 3 days, although during periods of 29 C temperatures in 1974, uptake was completed within 8 hours. In October and November, infusions were completed within 4 days, although in November the trees were defoliated. The time required for pressure injection

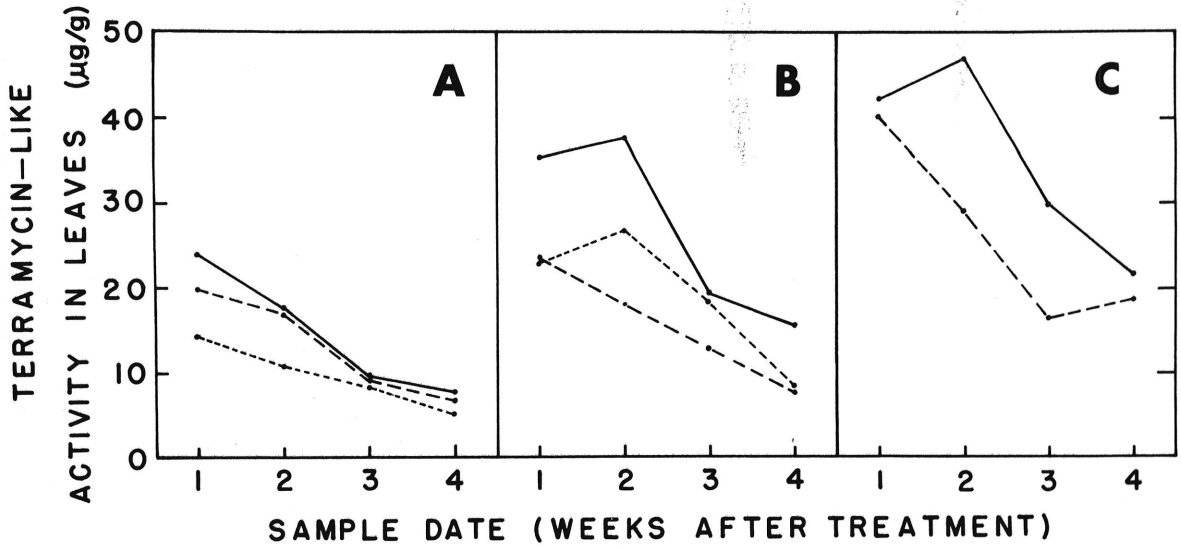

Fig. 1.-(A to C). Decline of terramycinlike activity (μg/g) in peach leaves following injections of: **A)** 1.25 g oxytetracycline-HCl (OTC) per tree; **B)** 2.5 g OTC per tree; and **C)** 3.75 g OTC per tree. Treatments were applied 12 to 13 September by infusion (•———•), by pressure injection (• — — —•), and as OTC concentrate (•— — —•).

varied considerably from tree to tree and usually exceeded 4 hours.

The foliage of injected trees turned slightly yellow for several weeks after treatment. Injections of 2.5 or 3.75 g OTC per tree in September also caused reddening of leaf veins after treatment and some dwarfing and yellowing of foliage the following spring (Table 1). September injections of 1.25 g OTC per tree caused a slight yellowing of leaves after treatment, but little or no yellowing appeared on spring foliage. Trees treated in October and November exhibited some death of branches the following spring, and many of the new leaves were chlorotic, strap-shaped, and small. Phytotoxicity ratings (Table 1) ranged from 3.2 to 4.8 for October and November treatments compared to 1.0 to 2.0 for most other treatments. Usually the chlorosis disappeared from treated trees by mid-June, but some trees treated in October and November remained stunted through early August.

Decline of TLA in leaves during the 4 weeks following treatment on 12 to 13 September was nearly linear for trees pressure-injected with 2.5 or 3.75 g OTC per tree and for trees injected with 1.25 g OTC by any method [Fig. 1-(A to C)]. For trees treated with 2.5 g OTC by infusion or concentrate (Fig. 1-B), or with 3.75 g OTC by infusion (Fig. 1-C), TLA in leaves did not decline until after the second sampling period. Most treatments had no detectable TLA in leaves after about 40 weeks (19 June 1975). For example, mean TLA in leaves of trees treated with 2.5 g OTC by infusion was 35.5, 15.1, 1.0, and 0.33 μg g after 1, 4, 37, and 40 weeks, respectively.

The mean TLA in leaves during autumn and/or spring was determined for each treatment (Table 1), and also for each rate, date, and method of application (Table 2 and 3). Effects and interactions of treatment factors were determined using several 3 × 2 split-plot factorial analyses in which the main treatment factors were split across four fall or two spring leaf-sampling dates. Treatment numbers from Table 1 are used to refer to the treatments included in the following statistical analyses.

In a comparison of infusion and pressure injection at 1.25, 2.5, and 3.75 g OTC per tree (treatments 1-6), high TLA in leaves was produced by high OTC rates (Table 2). The infusion method, when compared across all three rates, resulted in significantly ($P = 0.05$) greater TLA in leaves than did pressure injections. No rate × method interaction was detected.

In comparisons among treatment dates (treatments 4, 5, and 13-16), trees treated with 1.25 or 2.5 g OTC in September or with 1.25 g OTC in October showed low TLA in spring foliage (0.41-1.35 μg/g), whereas trees treated in November or with 2.5 g OTC in October showed higher levels (3.14-3.68 μg/g) (Table 3). The rate × date interaction was not significant.

The effects of solution concentration were tested by using three volumes of solution for pressure injections of two rates of OTC (treatments 4, 5, and 7-10). No significant differences in TLA were found among treatments involving 1.89, 3.79, and 7.58 liters of solution. When compared across the three volumes, trees treated with 2.5 g of OTC had significantly ($P = 0.05$) higher TLA in leaves than trees treated with 1.25 g of OTC.

The effect of solution concentration was tested again in 1975 when both the pear and the palm formulations of OTC were applied as concentrate or by infusion at 1.25 g/tree in final volumes of 10 ml, 0.94 liters, and 3.79 liters. These volumes resulted in mean TLA of 5.6, 12.8, and 10.9 μg/g, respectively, with LSD ($P = 0.05$) = 3.17. Formulation did not affect activity in leaves of 1975 treatments.

Residue analysis of fruit samples showed 0.032 and 0.030 μg TLA/g of fresh fruit for 1.25- and 2.5-g OTC treatments applied September 1973. Most of the September 1974 treatments, including those applied to trees also treated in 1973, resulted in no detectable fruit residue (<0.0125 μg/g). Infusion of 3.75 g OTC in September resulted in the highest level of TLA that was detected in fruit, 0.0255 μg/g.

The majority of treated trees showed no external signs of trunk damage. One year after treatment most holes had healed although small *Cytospora* infections occasionally were observed. However, trees treated with 2.5 g OTC as a concentrate (17 ml) showed extensive necrosis extending above and below some injection holes, and incidence of

TABLE 2. Terramycinlike activity (TLA) in peach leaf samples as influenced by two injection methods and three rates of oxytetracycline-HCl (OTC)

Rate of OTC (g/tree)[a]	Application method		Mean TLA for rates (μg/g)[c]
	Infusion	Pressure	
1.25	14.74[b]	13.21	13.97
2.50	26.98	15.63	21.31
3.75	35.24	26.21	30.72
Mean TLA for methods (μg/g)[d]	25.66	18.35	

[a]With both methods, all rates of OTC were applied in 3.79 liters of water on 9 September 1974.

[b]Each treatment mean represents the average TLA (μg/g) in leaves from four replicates sampled on four dates. LSD ($P = 0.05$) = 11.12.

[c]Least significant difference between means for application rates ($P = 0.05$) = 7.86.

[d]Least significant difference between means for application methods ($P = 0.05$) = 6.42.

TABLE 3. Terramycinlike activity (TLA) in peach leaf samples collected in spring 1975 as influenced by injection date and rate of oxytetracycline-HCl (OTC) applied

Treatment date[a] (1974)	Rate of OTC (g/tree)		Mean TLA for dates (μg/g)[c]
	1.25	2.50	
13 September	0.41[b]	0.68	0.55
10 October	1.35	3.58	2.46
5 November	3.14	3.68	3.41
Mean TLA for rates (μg/g)[d]	1.63	2.65	

[a]All treatments were applied by gravity infusions of 3.79 liters of solution.

[b]Each treatment mean represents the average TLA (μg/g) in leaves from four replicates sampled on two dates. LSD ($P = 0.01$) = 1.75.

[c]Least significant difference between means for dates ($P = 0.01$) = 1.24.

[d]Least significant difference between means for rates ($P = 0.01$) = 1.01.

Cytospora canker was higher than for other treatments.

DISCUSSION

This study indicates that a single injection of 1.25 or 2.5 g OTC per tree in September will give remission of X-disease for 1 year in medium-size peach trees. The failure of injections of 0.5 and 0.9 g OTC per tree to give year-long remission of symptoms is consistent with the results of a previous study (18) in which rates approaching 1.0 g OTC per tree gave a maximum of 77% X-disease symptom remission. Nyland (13) reported year-long remission of X-disease symptoms with less than 0.5 g OTC per tree, but he treated trees in both autumn and spring.

We assayed TLA in leaves because the potential effectiveness of various treatments for X-disease control should be reflected by their relative residual activity. Terramycinlike activity in leaves reflects, among other factors, how effectively the chemical is translocated into tree crowns. However, TLA in leaves could not be related quantitatively to symptom remission because most of the rates we tested provided a high degree of remission. Leaf residues were related to phytotoxicity in that residues and phytotoxicity increased together. Based on TLA in leaves, infusion was the most effective method for introducing OTC into infected peach trees.

The injection methods differed in ease of application. Infusion required 2-7 days to complete. With the pressure system, establishing pressure-tight connections was a problem, a significant amount of solution sometimes was lost by exudation through wounds and pruning cuts, and injections were not always finished in 1 day. Application of concentrated OTC required less equipment, and treatments were applied and holes sealed during one visit to the orchard.

Except for concentrated 10 ml injections, the volume of solution injected by infusion or pressure, did not significantly affect levels of TLA in leaves. By using higher solution concentrations and less volume per tree, treatment time may be reduced with no loss of effectiveness.

Treatment with concentrated OTC at the lower rate (1.25 g/tree in 10 ml) resulted in the lowest TLA of any treatment. But 2.5 g OTC per tree in 17 ml caused unacceptable damage to the tree trunks. Sands and Walton (18) did not mention trunk damage, but tested only 7 and 10% OTC solutions. They also used a different formulation which may have been less phytotoxic than those used in our study.

The relation of tree size to amount of OTC required has been defined to some extent. A dose of 1.25 g OTC produced symptom remission for 1 year in trees with trunk diameters up to 17 cm, and 2.5 g OTC was not damaging to trees with trunk diameters as small as 9 cm. In treatments not reported here, objectionable toxicity to foliage resulted from September infusion of 2.5 g into a tree of 7.5-cm trunk diameter, and trees with trunks of about 20-cm diameter developed a few symptoms 1 year after treatment with 1.25 g OTC. To be assured of year-long symptom remission in trees with trunk diameters greater than 17 cm, our experience indicates they should be treated with 2.5 g OTC using four or five injection sites.

The most unexpected result was the severe phytotoxicity of some October and all November treatments. Tetracyclines inhibit protein synthesis (5). Possibly some of the chemical injected in late autumn was stored in the tree and, in spring, moved to the new growth where even the low concentrations of OTC were detrimental to synthesis and development of new leaves. Trees were no longer growing when treated in September and the foliage could tolerate OTC concentrations 10 times greater than those which caused phytotoxicity in spring foliage. Chemical residues in leaves initially were high following September injections, but declined to below toxic levels by the following spring.

Another explanation for the toxicity of late autumn treatments is that trees treated at or after leaf fall may have concentrated the OTC in dormant buds where it damaged proplastids. The resultant production of defective plastids could explain the persistance of toxic symptoms on leaves after TLA no longer was detectable. Assays for TLA in dormant buds would have been helpful in assessing this theory.

Another advantage of September or postharvest treatments was that the fruit were not harvested for 9 to 11 months, thereby reducing the likelihood of unacceptable fruit residues. Residues were not detected in fruit from trees treated with 1.25 g OTC the previous September. Moreover, the level of residue in fruit from trees treated with 3.75 g was well below the desired tolerance level of 0.1 μg/g.

Because most treatments we tested were effective, the final choice of OTC rate and method for treating X-diseased peaches depends on equipment available, on tree size, and on preferences of the applicator. For best results, we suggest that injections be made after harvest but before normal leaf activity declines and at a rate of 1.25 g OTC per tree for all but small (possibly younger than 4 years old) and large trees. Trees with trunk diameters exceeding 17 cm may be injected with 2.5 g OTC per tree. For infusions or pressure injections the chemical should be mixed to apply 0.89 to 3.79 liters of solution per tree, and at least three holes per tree should be used. More holes are required to apply 1.25 g in 10-15 ml with the concentrate method. Even with appropriate treatment, trees infected with X-disease for several years require 1-2 years for new growth to replace the fruit-bearing wood killed by the disease.

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