Studies on the Therapeutic and Insect Vector Control of the Greening Disease of Citrus in India

T.K. Nariani and K.N. Bhagabati

The finding by Lafleche and Bové (1970) that greening disease of citrus is probably caused by a mycoplasmalike organism (MLO) has resulted in research on the use of tetracycline and other chemotherapeutants for the control of the disease (Capoor and Thirumalachar, 1973; Martinez *et al.*, 1970; Nariani, *et al.*, 1971; Nariani *et al.*, 1975; Raychaudhuri *et al.*, 1974; Schwarz *et al.*, 1974; Schwarz and van Vuuren, 1971; van Vuuren *et al.*, 1977). The present paper reports the results of experiments conducted with some tetracycline antibiotics, a chemotherapeutant, and a systemic fungicide on the expression of greening symptoms. It also reports the efficacy of controlling the psylla vector *Diaphorina citri* Kuway by systemic insecticides.

MATERIALS AND METHODS

Three tetracycline antibiotics. Terramycin (oxytetracycline hydrochloride, 20 per cent active ingredient, Pfizer and Co., Limited) ledermycin (demeclocycline hydrochloride, Cyanamid India, Limited); streptocycline (streptomycin 9 parts and chlortetracycline 1 part, Hindustan Antibiotics, Pimpri, Poona); a chemotherapeutant BP-101 (Hindustan Antibiotics, Pimpri, Poona); and the systemic fungicide Benlate (benomyl, E. I. duPont de Nemours and Co., U.S.A.) were tested. Four different methods of application of the chemicals were tried: (a) spraying with aqueous solutions, (b) dip treatment of greening-affected bud sticks, (c) branch injection using plastic funnels, and (d) trunk injections by gravity from plastic bags.

Four systemic insecticides: Rogor (dimethoate, Tata Fison, Limited); Metasystox (methyl demeton, Bayer India, Limited); Dimecron (phosphamidon) and Nuvacron (monocrotophos, both of Ciba Limited) were used as foliar sprays.

RESULTS

Effect of aqueous sprays. Artificially inoculated Mosambi seedlings (about 1½-2 years old) with greening symptoms were sprayed to run-off with the chemicals listed above using a hand sprayer. Sprays were applied weekly for 8 weeks inside the glasshouse. Observations were recorded on symptom expression (table 1).

Plants sprayed with terramycin, ledermycin, or streptocycline at 1000 ppm or BP-101 at 500 ppm recovered completely from greening symptoms. Plants sprayed with terramycin at 500 ppm, ledermycin at 100 or 500 ppm, and BP-101 at 100 ppm showed recovery from the disease symptoms in a majority of cases, whereas plants sprayed with streptocycline at 100 or 500 ppm, terramycin at 100 ppm, or Benlate at all three concentrations tested showed no recovery. Where recovery was observed it was temporary, and symptoms reappeared after varying periods (table 1).

Effect of dip treatment on greening-affected budsticks. Budsticks from Mosambi plants infected with greening were immersed in solutions of the chemicals at various concentrations for 3 hours. Control budsticks were immersed in tapwater for the same period. Treated and control budsticks were side-grafted on test plants to determine if the chemicals inhibited the pathogen.

Terramycin, ledermycin, streptocycline, or BP-101 at 1000 ppm were effective in inhibiting the greening pathogen completely when the infected budsticks were immersed in the chemi-

TABLE 1
RESULTS OF SPRAYING WITH TERRAMYCIN, LEDERMYCIN, STREPTOCYCLINE, BP-101, AND BENLATE ON GREENING-INFECTED MOSAMBI SEEDLING PLANTS

Treatments (ppm)	No. of plants treated	No. of plants showing recovery from symptoms		Intensity of disease symptoms*	Remarks
		Complete	Par-tial		
Terramycin					
100	5	0	0	++	
500	5	4	1	++	
1000	5	5	0	—	Symptoms reappeared in 10 weeks†
Ledermycin					
100	5	3	1	++	
500	5	4	1	++	
1000	5	5	0	—	Symptoms reappeared in 14 weeks
Streptocycline					
100	5	0	0	++++	
500	5	0	0	++++	
1000	5	4	0	—	Symptoms reappeared in 8 weeks
BP-101					
100	5	3	2	++	
500	5	5	0	—	Symptoms reappeared in 16 weeks
1000	5	5	0	—	Symptoms reappeared in 24 weeks
Benlate					
100	5	0	0	++++	
500	5	0	0	++++	
1000	5	0	0	++++	
Control (Water)	5	0	0	++++	

* — = no symptoms, ++ = mild symptoms, ++++ = severe symptoms.
† After last spray.

cals for 3 hours at room temperature (25°C) (table 2). With BP-101, only two of five test plants showed complete inhibition, and Benlate was not effective.

Effect of branch injection. This experiment was conducted with streptocycline only, because experiments with achromycin, ledermycin, and BP-101 have already been reported. Four, nearly uniform infected branches were selected on a 10-year-old grapefruit tree in the orchard. At the base of each branch, a hole was bored with a drill at a 45° angle about 1½ cm deep. A plastic funnel was inserted in the hole, and the outside of the funnel was sealed with paraffin wax to avoid leakage. The chemical (1 g/100 ml water) was poured into the plastic funnel in three branches, the fourth branch (control) was left untreated. After the desired quantity of the chemical was absorbed by the branch, the plastic funnel was removed and the hole plugged with paraffin wax and covered with cellophane tape. All three branches treated with 1 g streptocycline in solution showed partial recovery from symptoms. Severe symptoms reappeared on the treated plants after about 8 weeks (table 3).

Trunk injection by gravity from plastic bags. Mandarin trees, about 6 to 8 years old and infected with greening, were fed with antibiotic and other chemical solutions by gravity from plastic bags. Holes 2 to 2.5 cm deep were bored in trunks of the trees with a drill at a 45° angle to the trunk. The end of the polyethylene tube of the bag, which was made of hard plastic, was inserted in the hole. The bag was hung higher than the hole with a rope. The chemicals, at desired strengths, were poured into the top of the bag and were allowed to infuse into the plant by gravity.

Trees treated with 1 g of terramycin, ledermycin, or streptocycline, or 250 ml of 1000 ppm solution of BP-101 through gravity feeding bags showed temporary suppression of greening symptoms, whereas Benlate had no effect (table 4).

Efficacy of systemic insecticides on control of psylla. Insecticides at desired percentages and strengths (active ingredients) were sprayed with a foot sprayer at weekly intervals for 4 weeks during March, when psylla breeds actively on the new flushes. Psylla counts were recorded at random before and after each spray on 100 leaves from each tree. Observations on psylla populations were continued at weekly intervals for 6 weeks after the last spray to determine the residual effect of the insecticides. Psylla populations on the orchard trees decreased, even after the first spray, in all treatments except the control (table 5). The populations gradually decreased after each additional spray. Spraying with 0.1 or 0.2 per cent Rogor (0.03 or 0.06 per cent dimethoate), 0.1 or 0.2 per cent Dimecron (0.085 or 0.17 per cent phosphamidon) resulted in 100 per cent mortality after the third spray, whereas Metasystox at 0.05 per cent (0.012 per cent methyl demeton) or Nuvacron at 0.05 per cent (0.02 per cent monocrotophos) produced 100 per cent mortality after the fourth spray. The residual effect of 0.1 or 0.2 per cent Rogor, 0.5 per cent Metasystox, 0.2 per cent Dimecron, or 0.05 per cent Nuvacron lasted for 4 weeks after the last spray, whereas the residual effect of Dimecron at 0.1 per cent lasted for only 3 weeks.

DISCUSSION AND CONCLUSIONS

The results indicate that, in addition to achromycin, ledermycin, and BP-101 reported earlier (Capoor and Thirumalachor, 1973; Martinez *et al.*, 1970; Nariani *et al.*, 1971; Nariani *et al.*, 1975; Raychaudhuri *et al.*, 1974; Schwarz *et al.*, 1974; Schwarz and van Vuuren, 1971; van Vuuren *et al.*, 1977) terramycin and streptocycline have a suppressive effect on symptoms when applied by foliar spray or trunk injections. Temporary suppression of greening symptoms was also observed when streptocycline solution was infused into the branches through plastic funnels.

In the dip treatment experiments, budsticks immersed in terramycin, ledermycin, streptocycline, or BP-101

at 1000 ppm for 3 hours were freed from the greening pathogen completely, whereas these chemicals at 100 or 500 ppm were not effective. Martinez *et al.* (1970), using achromycin at 1000 ppm solution, also reported no leaf symptoms in propagations from budsticks immersed for 25 minutes.

Benlate, a systemic fungicide which was reported by Raychaudhuri *et al.* (1972) to be effective against sandal spike, a disease associated with MLO, was not effective against greening disease of citrus.

The insecticide control of *D. citri* can be achieved by four weekly sprays of 0.1 per cent Rogor (.03 per cent dimethoate), 0.05 per cent Metasystox (.012 per cent methyl demeton), 0.1 per cent Dimecron (0.085 per cent phosphamidon), or 0.05 per cent Nuvacron (0.02 per cent monocrotophos). Catling (1969) and Buitendag (1972) effectively controlled *Trioza erytreae* del Guercio, vector of South African citrus greening, with concentrations of dimethoate and monocrotophos as low as 0.0025 per cent and 0.001 per cent, respectively.

TABLE 2
RESULTS OF 3-HOUR DIP TREATMENT OF GREENING-AFFECTED BUDSTICKS IN CHEMICAL SOLUTIONS

Treatment* (ppm)	No. of plants showing infection		Intensity of disease symptoms†	Back inoculation test
	Complete	Partial		
Terramycin				
100	4	1	++++	
500	2	3	++	
1000	0	0		—
Ledermycin				
100	3	2	++++	
500	2	3	++	
1000	0	0		—
Streptocycline				
100	5	0		
500	2	3	++++	
1000	0	0		—
BP-101				
100	2	3	++	
500	2	1	++	—
1000	0	0		—
Benlate				
100	5	0		
500	5	0		
1000	5	0		
Control (water)	5	0		

* Five budsticks used for each treatment.

† Each of 5 treated sticks propagated by grafting to healthy rootstock; ++ = mild symptoms, ++++ = severe symptoms, — = no symptoms.

TABLE 3
RESULTS OF BRANCH INJECTION OF GREENING-AFFECTED GRAPEFRUIT TREES WITH STREPTOCYCLINE

Treatment	No. branches treated	No. branches showing recovery from symptoms	Intensity of disease symptoms*
Streptocycline (1 g/100 ml water)	3	3	++†
Control (untreated)	1	0	++++

* ++= mild symptoms, ++++ = severe symptoms.
† Symptoms reappeared after 8 weeks.

TABLE 4
RESULTS OF TRUNK INJECTION OF THE GREENING-AFFECTED MANDARIN TREES WITH CHEMICALS BY GRAVITY FROM PLASTIC BAGS

Treatment	No. plants treated	No. plants showing recovery	Intensity of disease symptoms*
Terramycin (1 g/250 ml)	2	2	++†
Ledermycin (1 g/250 ml)	1	1	++†
Streptocycline (1 g/250 ml)	1	1	++†
BP-101 (250 ml at 1000 ppm)	1	1	++†
Benlate (1 g/250 ml)	1	0	++++
Control (water)	1	0	++++

* ++ = mild symptoms, ++++ = severe symptoms.
† Symptoms reappeared.

TABLE 5
EFFECT OF DIFFERENT SYSTEMIC INSECTICIDES ON CITRUS PSYLLA POPULATION ON ORCHARD TREES
(NUMBER OF PSYLLA PER 100 LEAVES)

	Spray applications, 1977													
	Mar. 5		Mar. 12		Mar. 19		Mar. 26		Weeks after last treatment					
Treatments	Pre	Post	Pre	Post	Pre	Post	Pre	Post	1	2	3	4	5	6
Dimethoate														
0.06%	1126	783	732	360	132	60	nil	nil	nil	nil	nil	nil	5	11
0.03%	1237	993	803	483	280	80	nil	nil	nil	nil	nil	nil	8	13
0.15%	1003	832	730	612	372	130	85	12	8	21	25	33	52	53
Methyl demeton														
0.012%	1380	863	639	410	270	80	33	nil	nil	nil	nil	nil	28	32
0.005%	1103	873	732	532	400	150	68	23	18	35	40	48	55	69
0.0025%	1073	899	782	580	465	180	75	32	24	40	49	55	62	75
Phosphamidon														
0.17%	1273	772	739	672	160	50	nil	nil	nil	nil	nil	nil	12	18
0.085%	1384	893	832	580	180	72	nil	nil	nil	nil	nil	8	30	38
0.0425%	1282	902	840	586	230	103	35	18	12	24	35	65	68	73
Monocrotophos														
0.02%	1300	983	863	573	180	90	12	nil	nil	nil	nil	nil	19	25
0.012%	1079	832	772	535	200	115	38	21	20	30	36	43	58	63
0.008%	1283	830	789	600	312	173	48	28	28	38	43	60	72	81
Control	1330	1430	1430	1389	1360	1343	1315	1300	1280	1280	1277	1235	1168	1163

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