The Responses of the Likubin Pathogen to Antibiotics and Heat Therapy

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Likubin is the most destructive widespread disease of citrus trees in Taiwan. A mycoplasmalike organism (MLO) has been found associated with the disease (Su, 1970) and the MLO etiology has been further indicated by the therapeutic effect of tetracyclines (Su and Leu, 1972). The disease has been assumed to be closely related to the greening group of MLO diseases. Bové *et al.* (1974) predicted that likubin would belong with citrus decline and leaf mottling, to the heat-tolerant group of greening diseases. Martinez *et al.* (1970) demonstrated symptom suppression of citrus greening by a foliar spray of 100 ppm tetracycline-HCl and obtained greening-free budsticks by immersion in 1,000 ppm tetracycline-HCl for 25 minutes before bud inoculation. The incidence of fruit greening was reduced by trunk injection of 500 or 750 ppm tetracycline-HCl in severely affected Valencia orange trees (Schwarz and van Vuuren, 1971). Su and Leu (1972) reported that 100 ppm of achromycin completely suppressed symptom development of likubin-affected scions dip-treated for 15 hours, while aureomycin (chlortetracycline) showed a little therapeutic effect. More recently, Schwarz *et al.* (1974) and Capoor *et al.* (1974) tried to control citrus greening by trunk injections, dry applications under bark and foliar sprays with tetracyclines and some other chemotherapeutants. Su and Leu (1972) easily destroyed the likubin pathogen by dipping affected budsticks in hot water at 48 to 54℃ for 10 minutes.

The experiments reported here were done to clarify the response of the likubin pathogen in affected citrus trees to tetracycline transfusion and to heat-therapy in a hot-air chamber. An attempt was also made to find other therapeutants effective against likubin.

EXPERIMENTS AND RESULTS

Dip treatment of diseased scions. In earlier work (Su and Leu, 1972), dipping likubin-affected Ponkan budwood in 100 ppm achromycin for 15 hours completely suppressed symptom development in scions propagated on Rangpur lime rootstock. Aureomycin at 100 ppm had less chemotherapeutic effect than achromycin. The present study was made to confirm the effectiveness of oxytetracycline-HCl (terramycin). Table 1 illustrates the effect of tetracycline immersion on symptoms and growth of the scions. Shoots grown from almost all of the diseased buds treated with 50 or 100 ppm of tetracycline showed normal growth with no symptoms in Ponkan mandarin, Leuchen sweet orange and Tankan, while all diseased buds treated with water produced poor shoots with typical symptoms. Terramycin was almost as effective as achromycin but was slightly inferior in Ponkan and Tankan, which had moderate growth and mild symptoms on one-fourth of the test scions.

The results of another dipping test, employing chemicals used for animal or human respiratory mycoplasmosis, are shown in table 2. In this test on budwood of sweet orange, Ponkan, Tankan, and Satsuma, achromycin suppressed symptoms and caused no visible phytotoxicity, whereas none of the other test chemicals was highly effective. Some scions treated with penicillin G or chloramphenicol produced healthy-looking shoots having a moderate growth rate, and others with severe or mild symptoms made poor growth. Ridzol-s (histomonostaticum) and dynamycin might be slightly effective against the pathogen, as indicated by more new shoots with mild symptoms on

TABLE 1
EFFECT OF DIP TREATMENT WITH TETRACYCLINES ON SYMPTOM DEVELOPMENT IN LIKUBIN-AFFECTED BUDWOOD

Treatments*	Symptom development†	Scions treated/ scions showing symptoms	Scion growth
Sweet orange (Leuchen)			
Achromycin	–	4/0	Good
Terramycin	–	4/0	Good
Water check	+	4/4	Poor or dead
Mandarin (Ponkan)			
Achromycin	–	4/0	Good
Terramycin	–, ±	4/1	Good or moderate
Water check	+	4/4	Poor or dead
Tankan			
Achromycin	–	4/0	Good
Terramycin	–, ±	4/1	Good or moderate
Water check	+	4/4	Poor or dead

*Tetracycline concentration was 100 ppm.
† – = no symptoms; + = mild symptoms; + = distinct symptoms.

TABLE 2
EFFECT OF DIP TREATMENT OF LIKUBIN-AFFECTED BUDWOOD WITH VARIOUS MATERIALS ON SYMPTOM DEVELOPMENT IN CITRUS SCIONS

Treatments*	Symptom development†	Scions treated/ scions showing symptoms	Scion growth
Sweet orange			
Achromycin	–	4/0	Good
Penicillin G	±, –	4/2	Moderate
Dynamycin	±	4/4	Moderate
Ridzol-S	+	4/4	Poor or dead
Water check	+	4/4	Poor or dead
Ponkan			
Achromycin	–	4/0	Good
Terramycin	–, ±	4/1	Good or moderate
Penicillin G	±, –	4/2	Moderate
Ridzol-S	±	4/4	Moderate or poor
Chloramophenicol	+, –	4/3	Moderate or poor
Tylan	+	4/4	Poor or dead
Water check	+	4/4	Poor or dead
Tankan			
Achromycin	–	3/0	Good
Penicillin G	+, –	3/2	Moderate or poor
Water check	+	3/3	Poor or dead
Satsuma			
Achromycin	–	4/0	Good
Water check	+	4/4	Poor

*Concentration of the test chemicals, except penicillin G at 200 IU/ml, was 100 ppm.
† – = no symptoms; ± = mild symptoms; + = distinct symptoms.

treated than on untreated scions. Tylan (tylosin tartrate) was ineffective. The results of the dip trials indicate only oxytetracycline-HCl and achromycin to be effective therapeutants for curing likubin-diseased budsticks.

Tetracycline transfusion. In preliminary foliar applications, no remarkable response was noted on affected trees sprayed with a 100 ppm achromycin. A modification of the transfusion technique used by Nyland and Moller (1973), for treating pear decline was used to inject field trees with achromycin or terramycin. Diseased Ponkan and Tankan trees on Sunki or Rangpur lime rootstock were treated by transfusing tetracycline solutions into the trunks at various dosages from a plastic reservoir through rubber tubes connected to aluminum or brass fittings inserted into small holes (5.9 x 30 mm) in the tree trunks (fig. 1). Symptoms in trees of the 11-year-old Ponkan/Sunki combination injected with various dosages of achromycin at different concentrations in late winter were read nine months after transfusion. More than two thirds of the diseased Ponkan trees transfused with 300 to 400 ml of 1,000 ppm achromycin solution per tree appeared completely cured. These treatments resulted in 65 to 88 per cent apparently healthy new shoots (table 3). Less than one quarter of the trees injected with 200 ml of 1,000 ppm or 400 to 800 ml of 500 ppm achromycin showed nearly complete recovery. Accordingly, a dosage of 300 to 400 ml of 1,000 ppm tetracycline solution may be recommended for treating likubin-affected trees about 10 years old.

Table 4 shows results of a field trial on the effect of season of application and pruning on tree recovery following transfusion with achromycin. Ponkan and Tankan trees were injected with 300 ml of 1,000 ppm tetracycline-HCl. Application during the March flush period gave better control of likubin in Tankan trees than the December application. The recovery index was 75 to 98 per cent for the spring

Fig. 1. Transfusion apparatus consisting of a 2-liter plastic reservoir, 7 mm rubber tubes and aluminum tube connector with dimensions of 6 × 13 + 8 × 5 + 7 = 13 (upper right). Manual drill was used for making three holes in the trunk.

TABLE 3
SYMPTOM SUPPRESSION IN LIKUBIN-AFFECTED CITRUS TREES BY TRANSFUSION WITH VARIOUS DOSAGES OF ACHROMYCIN

Concentration ppm	Dosage ml	Total Trees treated	Trees having indicated per cent of healthy new shoots			Recovery index* per cent
			100-90	89-40	< 40	
1,000	200	4	1	1	2	47.5
1,000	300	6	4	1	1	65.1
1,000	400	4	3	1	0	87.5
500	400	4	1	2	1	50.0
500	600	4	0	3	1	42.5
500	800	4	0	2	2	40.0

*Healthy new shoots/total new shoots x 100.

TABLE 4
SYMPTOM SUPPRESSION OF LIKUBIN-AFFECTED CITRUS TREES BY ACHROMYCIN TRANSFUSION WITH PARTICULAR REFERENCE TO SEASON OF APPLICATION AND PRUNING

Treatment	Total trees treated	Trees showing indicated percentages of healthy new shoots			Recovery index* per cent
		100-90	89-40	< 40	
Tankan					
Winter application					
Without pruning	5	1	3	1	50
Pruned	4	3	1	0	90
Spring application					
Without pruning	4	2	1	1	75
Pruned	6	5	1	0	98
Ponkan					
Winter application					
Pruned	5	4	1	0	94
Spring application					
Without pruning	5	1	2	2	50
Pruned	5	4	1	0	84
Total	34	20 (59%)	10 (29%)	4 (12%)	

*Healthy new shoots/total new shoots x 100.

and 50 to 90 per cent for the winter application. However, Ponkan trees responded better to the winter than to the spring application. Pruning improved the effectiveness of the transfusion significantly regardless of the season of application of tetracycline. Pruned trees produced normal new growth but the untreated trees continuously showed yellowing of old leaves, small chlorotic leaves on new shoots, defoliation and dieback (figs. 2 and 3). Some severely affected citrus trees were pruned heavily after transfusion treatment and re-

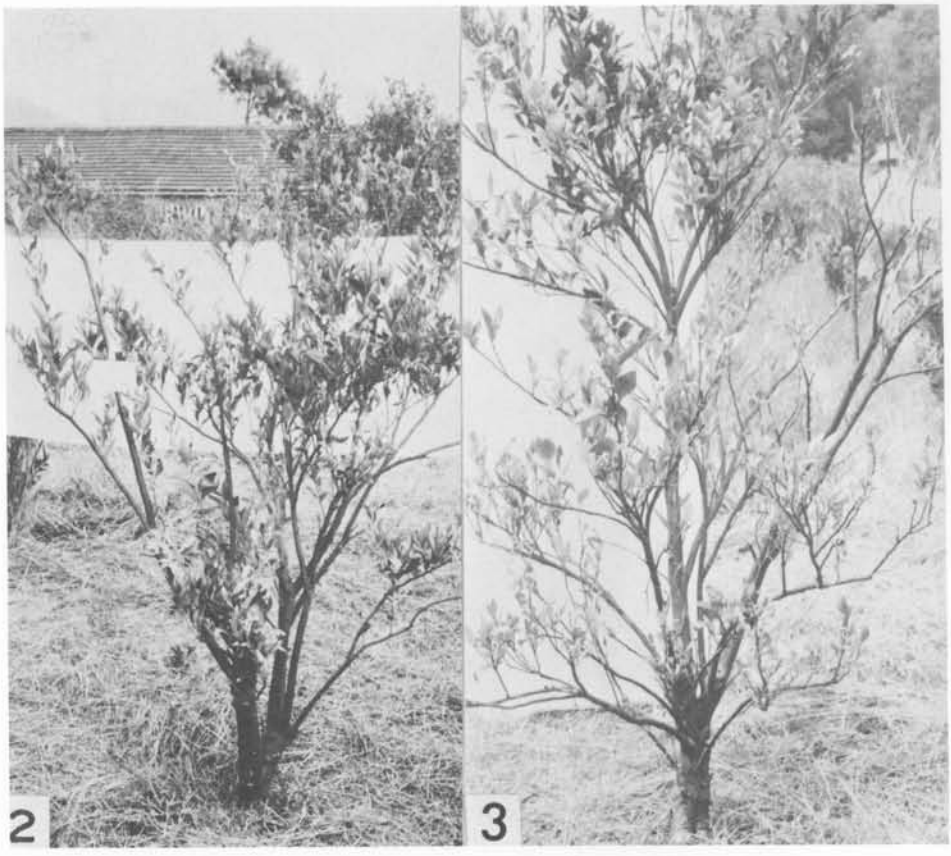

Fig. 2. Tankan tree treated with 300 ml of 1,000 ppm achromycin solution and pruned just after transfusion in the early spring (March) showed complete recovery from the disease by producing healthy shoots six months after transfusion.

Fig. 3. An untreated 8-year-old Tankan affected with likubin continually showed severe symptoms: leaf yellowing, small chlorotic leaves, defoliation, and dieback.

sponded well (fig. 4). The luxuriant growth of healthy spring flushes was frequently observed on pruned branches of the treated citrus trees (fig. 5). New shoots with chlorosis and stunting symptoms were frequently found near a terminal diseased twig on an unpruned branch of a treated tree (fig. 6). The closer the shoots were to the trunk, the healthier they looked. About 60 per cent of the treated trees in this experiment completely recovered, 30 percent showed partial recovery and 10 per cent were unchanged.

The effectiveness of oxytetracycline HCl and tetracycline HCl were compared in a field trial, using Ponkan trees 11 years old. Oxytetracycline-HCl was as effective as achromycin. About 80 per cent of the trees transfused with 300 ml of 1,000-ppm oxytetracycline developed no symptoms although some very slender green leaves were found on new shoots produced after transfusion (fig. 7). This malformation of Ponkan leaves was assumed to be a side-effect of oxytetracycline. Twigs with malformed leaves produced normal leaves in subsequent

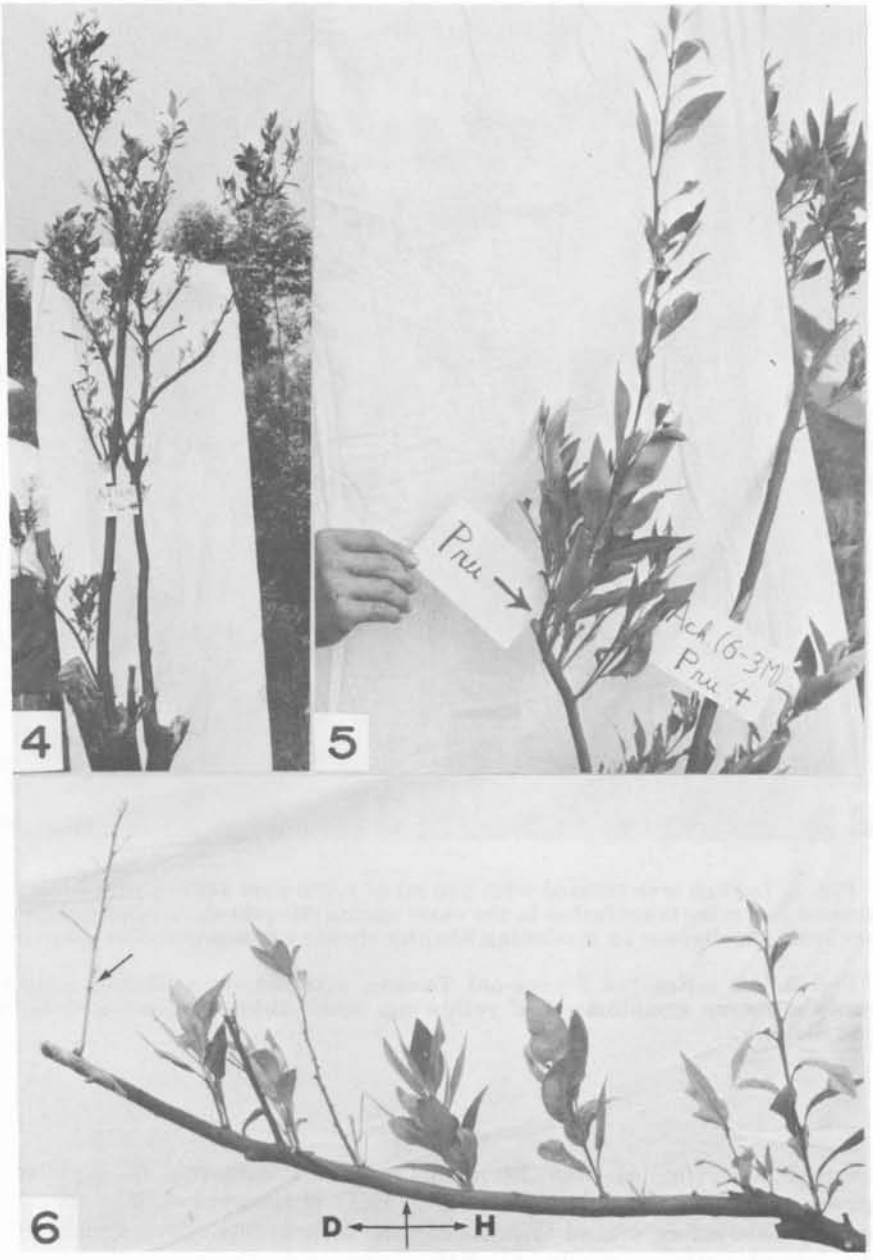

Fig. 4. Severely affected Tankan tree 11 years old showing recovery by producing healthy new shoots on heavily pruned branches six months after achromycin transfusion.

Fig. 5. Luxuriant growth of healthy new shoots on a pruned branch of the transfused Tankan tree shown in fig. 2.

Fig. 6. Unpruned branch of a transfused Tankan tree, showing symptoms in several shoots (D) growing near a diseased twig (arrow) on the branch tip. The closer the shoots were to the trunk, the healthier they appeared.

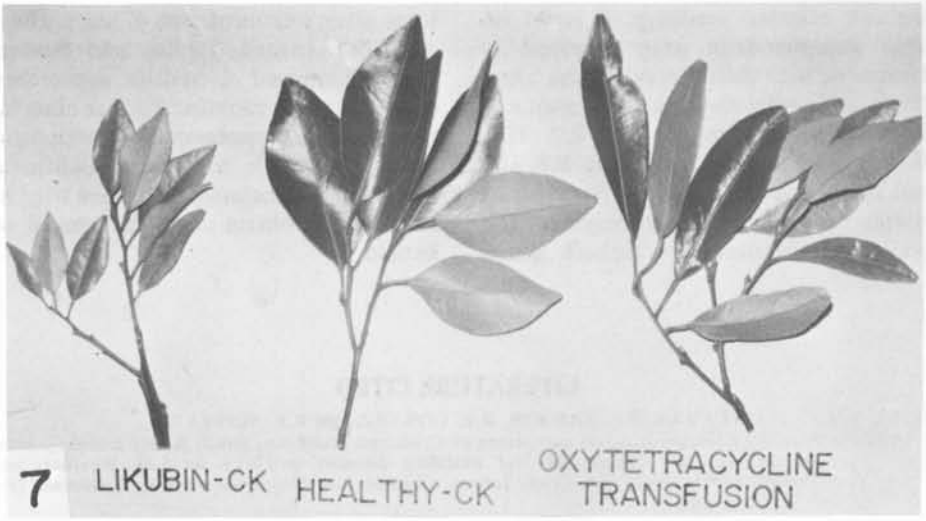

Fig. 7. Slenderizing side effect (right) on leaves of Ponkan tree transfused with oxytetracycline.

flushes. Application of soluble foliar fertilizer or liquid complete fertilizer seemed to improve the effectiveness of the transfusion treatment.

Heat treatment. In an earlier heat-therapy experiment (Su and Leu, 1972) the likubin pathogen was inactivated by dipping diseased scions in water at 48 to 54°C for 10 minutes. We treated diseased seedlings of Ponkan or sweet orange on Rangpur lime trees with hot air. Recovery from the disease was demonstrated on the affected seedlings heated at 40°C in a growth chamber for 10 days. However, no remission of symptoms occurred in test seedlings heated at the same temperature for three to seven days. Treatment more than 13 days injured the plants. Heat treatment at 42°C for one to three days failed to inactivate the pathogen and extension of heating to seven days caused injury. Diseased Ponkan and Tankan seedlings treated in a growth chamber at 30 and 40°C on an 8- and 16-hour cycle for one month showed remarkable recovery by producing healthy normal shoots.

DISCUSSION AND CONCLUSIONS

Achromycin at 100 ppm was the best material for dip treatment of budsticks (Su and Leu, 1972) and proved best for suppressing Philippines leaf mottling (Martinez, Nora, and Armedilla, 1970). In our field trials achromycin, as well as terramycin, applied by a modified transfusion technique adapted from Nyland and Moller (1973) were effective on likubin-affected Tankan and Ponkan trees during the spring flush. Schwarz, Moll, and van Vuuren (1974) injected 1 liter of 250 to 1,000 ppm solutions of these tetracyclines by means of a modified blowtorch injector, and reported that the greatest decrease in incidence of fruit greening was obtained with one liter of 1,000 ppm tetracycline-HCl solution per Valencia tree during the September (spring) flush.

Pruning off diseased twigs soon after transfusion considerably improved the effectiveness of our trunk injections with 1,000 ppm achromycin. Presumably, the transfused tetracycline was unable to diffuse to all the tips of diseased twigs due to low transpiration caused by the small diseased leaves on such twigs. The MLO remaining untreated in the terminal affected twigs might be eliminated by pruning the diseased twigs after transfusion. Capoor *et al.* (1974) found that

dieback affected seedlings of sweet orange and mandarin were improved by treatment with foliar sprays, trunk injections, and under-the-bark applications of achromycin, ledermycin and B.P. 101. Recovery of plants treated with B.P. 101 was better and longer lasting than that of plants treated with achromycin. The period to recurrence of dieback symptoms after treatment was 47 days. However, our treated Tankan and Ponkan trees maintained a healthy appearance more than nine months. Further observations for the reappearance of symptoms have been made and some additional application of materials have been tried in an effort to obtain complete control of likubin.

LITERATURE CITED

BOVÉ, J.M., E.C. CALAVAN, S.P. CAPOOR, R.E. CORTEZ, and R.E. SCHWARZ
1974. Influence of temperature on symptoms of California stubborn, South Africa greening, India citrus decline, and Philippines leaf mottling diseases, p. 12-15. *In* L.G. Weathers and M. Cohen, (eds.), Proc. 6th Conf. Intern. Organization Citrus Virol. Univ. California Div. Agr. Sci., Berkeley.

CAPOOR, S.P., M.J. THIRUMALACHAR, P.K. PANDEY, and N.K. CHAKRABORTY
1974. Control of the greening disease of citrus by B.P.-101: a new chemotherapeutant, p. 50-52. *In* L.G. Weathers and M. Cohen, (eds.), Proc. 6th Conf. Intern. Organization Citrus Virol. Univ. California Div. Agr. Sci., Berkeley.

MARTINEZ, A.L., D. M. NORA, and A. L. ARMEDILLA
1970. Suppression of symptoms of citrus greening disease in the Philippines by treatment with tetracycline antiobiotics. Plant Dis. Rep. 54: 1007-09.

NYLAND, G., and W.J. MOLLER
1973. Control of pear decline with a tetracycline. Plant Dis. Rep. 57: 634-37.

SCHWARZ, R.E., J.N. MOLL, and S.P. VAN VUUREN
1974. Control of citrus greening and its psylla vector by trunk injections of tetracycline and insecticides, p. 26-29. *In* L.G. Weathers and M. Cohen, (eds.), Proc. 6th Conf. Intern. Organization Citrus Virol. Univ. California Div. Agr. Sci., Berkeley.

SCHWARZ, R.E. and S.P. VAN VUUREN
1971. Decrease in fruit greening of sweet orange by trunk injection of tetracyclines. Plant Dis. Rep. 55: 747-50.

SU, H.J.
1970. Further study on the pathogen complex causing likubin of citrus. Plant Protection Bul. (China) 12: 190 (Abstract in Chinese).

SU, H.J., and S.C. LEU
1972. Study on the pathogen complex causing likubin of citrus in Taiwan. I. Nature of mycoplasmalike organism associated with the disease. Proc. National Science Council No. 5 p. 109-26.