Control of the Greening Disease of Citrus by B.P.-101: A New Chemotherapeutant

S. P. Capoor, M. J. Thirumalachar, P. K. Pandey, and N. K. Chakraborty

Die-back of citrus is widespread in India, and has been attributed to the pathogen of greening disease (*4, 5*) transmitted by the Oriental citrus psylla, *Diaphorina citri* (*2*). A mycoplasmalike organism was shown to be associated with the greening disease (*7*).

Greening-affected citrus trees show a variety of symptoms (*3, 11*), the severity of which has been amply emphasized. Spread of the pathogen in nature by an insect rules out control by use of disease-free budwood. While tetracycline antibiotics have been used to suppress symptoms of stubborn (*6*), leaf-mottle yellows disease (*9*), and greening (*10, 12*), the effect was not permanent, and the antibiotics are unstable (*8*). Recovered plants may still contain some mycoplasmalike bodies, which apparently multiply either when the concentration of antibiotics decreases in the plant system or treatment is stopped (*13, 14*).

We tested a new chemotherapeutant, B.P.-101, produced by the Hindustan Antibiotics Ltd., Pimpri, Poona, for the treatment of greening in lime (*3*), sweet orange, and mandarin trees. Results are reported in this paper.

MATERIALS AND METHODS

Test plants were inoculated with leaf-patch grafts or with buds. The source of greening was a pure culture maintained in a glasshouse, on sweet orange seedlings inoculated by *Diaphorina citri*. The source of tristeza virus was a pure culture of a severe strain of the virus maintained on lime seedlings in a glasshouse. All test plants were either grown from seed or propagated on Rough lemon rootstock in the glasshouse.

Achromycin (tetracycline hydrochloride) and ledermycin (demethyl chlortetracycline hydrochloride) were used in addition to B.P.-101. Application was by foliar spray, trunk injection, and injection under the bark, with either 500-ppm aqueous solution or dry chemicals at the rate of 0.25 to 1.50 gm per plant, as previously described (*3*). Treatments were repeated at suitable intervals, and continued for three months. Untreated were repeated at suitable intervals, and control plants were indexed for greening with Mosambi sweet orange (*2*) and for tristeza with Kagzi lime seedlings (*1*).

RESULTS

Treatment of seedlings. Four-year-old normal and greening-affected seedlings of Mosambi sweet orange and mandarin were treated with three chemicals by the three methods described. Seedlings were showing typical advanced symptoms of greening prior to treatment. Some seedlings sprayed with achromycin and ledermycin had recovered completely by 90 days following treatment. Recovery in plants sprayed with B.P.-101 was delayed. Some sprayed plants did not respond to treatment with any of the chemicals (table 1). Greening symptoms reappeared in the recovered seedlings within 60 days after treatments were discontinued. This test indicated that: (1) either the chemicals were not absorbed by the leaves or were not absorbed properly; (2) the effect of these chemicals was short-lived; or (3) the

TABLE 1
RESPONSE OF GREENING-AFFECTED CITRUS SEEDLINGS TO TREATMENT WITH B.P.-101 AND TETRACYCLINES

Treatment	Seedlings treated/seedlings recovered following treatment by:			Days to reversion to greening after treatment
	Foliar spray	Application under the bark	Injection in main stem	
Sweet orange:				
Achromycin	10/7	10/8	10/10	50
Ledermycin	10/5	10/7	10/8	33
B.P.-101	10/4	10/8	10/10	Still green after 270 days
Mandarin:				
Achromycin	5/2	5/3	5/3	47
Ledermycin	5/0	5/2	5/2	35
B.P.-101	5/0	5/3	5/4	Still green after 270 days

plants outgrew the effect of the chemicals as they put out new growth.

Application of the chemicals under the bark was more effective than were foliar sprays, but best results were obtained when chemicals were injected into stems of test plants (table 1). Application of achromycin and B.P.-101 by this method brought about recovery in all seedlings of sweet orange, while mandarin, for some reason not yet understood, did not respond (table 1). Recovery in plants treated with B.P.-101 was more complete and longer lasting than in plants treated with Achromycin. Plants treated with B.P.-101 continued to develop more chlorophyll in their leaves, and developed more healthy looking side shoots and terminal growth for a longer period.

Treatment of budded plants. Since all trees in orchards are found to be affected both by greening and tristeza, our test plants were inoculated with both pathogens to simulate field conditions. Plants of Mosambi sweet orange, grapefruit, Nagpur mandarin, and Kagzi lime were grown on Rough lemon, in a glasshouse. Plants were inoculated with tristeza virus 42 months later, and with the greening pathogen 52 months later. Within 31 months, all inoculated plants developed severe symptoms of the greening disease. Presence of tristeza virus was confirmed by indexing.

Eight plants each of sweet orange, grapefruit, mandarin, and lime were transferred to large pots outside the glasshouse. In November, 1971, six plants of each were treated with 10 ml of a 500-ppm aqueous solution of B.P.-101 by the injection method, and two of each were kept as untreated controls. Application of B.P.-101 was repeated twice at 30-day intervals.

All treated plants recovered completely within 190 days following B.P.-101 treatment, while untreated controls deteriorated. After 300 days, treated plants continued to develop more chlorophyll in their leaves, and also developed vigorous, healthy looking side shoots and terminal growth. Plants were not completely free of the greening pathogen since they gave positive indication of greening when indexed by leaf-patch grafting.

DISCUSSION AND CONCLUSIONS

Results of these experiments clearly show that B.P.-101 is superior to tetracycline antibiotics in producing faster and longer lasting recovery of greening-affected citrus plants. Treated plants, even though appearing normal, indexed positive for the greening organism. It will be necessary to estimate the rate of multiplication and growth cycle of the greening organism in plants, following application of B.P.-101, to determine treatment intervals.

LITERATURE CITED

1. CAPOOR, S. P.
1961. Kagzi lime: an indicator plant of the citrus decline virus in India. Indian Phytopath. **14:** 109–12.
2. CAPOOR, S. P., G. RAO, AND S. M. VISWANATH
1967. *Diaphorina citri* Kuway., a vector of the greening disease of citrus in India. Indian Jour. Agr. Sci. **37:** 572–76.
3. CAPOOR, S. P., AND M. J. THIRUMALACHAR
1973. Cure of greening-affected citrus plants by chemotherapeutic agents. Plant Dis. Reptr. **57:** 160–63.
4. FRASER, L. R., D. SINGH, S. P. CAPOOR, AND T. K. NARIANI
1966. Greening virus, the likely cause of citrus die-back in India. FAO Plant Protect. Bul. **14:** 127–30.
5. FRASER, L. R., AND D. SINGH
1968. Citrus die-back in India—the contribution of greening virus. *In:* Proc. 4th Conf. Intern. Organ. Citrus Virol. (J. F. L. Childs, ed.) Gainesville: Univ. Florida Press, pp. 141–44.
6. IGWEGBE, E. C. K.
1970. Studies on the nature and transmission of the causal agent of stubborn disease of citrus: Association of a mycoplasmalike organism with the disease. Ph.D. thesis, University of California, Riverside.
7. LAFLÈCHE, D., AND J. M. BOVÉ
1970. Structures de type mycoplasme dans les feuilles d'orangers atteints de la maladie du "greening." Compte. rend. Acad. Sci. Paris **270:** 1915–17.
8. MARAMOROSCH, K., R. R. GRANADOS, AND H. HIRUMI
1970. Mycoplasma diseases of plants and insects. Adv. Virus Res. **16:** 135–93.
9. 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 antibiotics. Plant Dis. Reptr. **54:** 1007–09.
10. NARIANI, T. K., S. P. RAYCHAUDHURI, AND S. M. VISWANATH
1971. Response of greening pathogen of citrus to certain tetracycline antibiotics. Current Sci. **40:** 552.
11. SCHNEIDER, H.
1968. Anatomy of greening—diseased sweet orange shoots. Phytopathology **58:** 1155–60.
12. SCHWARZ, R. E.,AND S. P. VAN VUUREN
1971. Decrease in fruit greening of sweet orange by trunk injection of tetracyclines. Plant Dis. Reptr. **55:** 747–50.
13. SINHA, R. C., AND E. A. PETERSON
1972. Uptake and persistence in aster plants and vector leafhoppers in relation to inhibition of clover phyllody agent. Phytopathology **62:** 377–83.
14. STARON, T., M. T. COUSIN, AND C. GRISON
1968. Action de quelques antibiotiques sur des maladies végétales du type "Jaunisse europeene." Compte rend. Acad. Sci. Ser. **D 267:** 2328–31.