Further Investigations of Likubin of Ponkan Mandarin

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Since 1970 the authors have studied likubin of Ponkan (Tanaka and Doi, 1974). Some results of these studies were presented at the First International Citrus Congress in 1973 (Tanaka, 1973). The present paper reports the results of additional electron microscopy, graft inoculations, and tetracycline treatment of diseased plants.

ELECTRON MICROSCOPY

Ultrathin sections of very young tissues of diseased Ponkan always showed mycoplasmalike bodies, presumably the likubin agent, in the phloem elements. These bodies were surrounded by a thick envelope composed of a double unit membrane, that is an inner and outer unit membrane. This envelope measured 12-23 nm, considerably thicker than single unit membranes usually found in other phytopathogenic MLO. Unlike rickettsia or bacteria, no R-layer was found in the electron transparent zone between the inner and outer unit membranes. Occasionally, very long filamentous particles, measuring 14,000 x 300 nm, were found. Some of these showed irregular constrictions or a loose spiral form. Every particle contained numerous ribosomes and nuclear net strands. Sometimes the particles appeared to be passing through sieve pores, revealing their elastic nature. The fine structure of the envelope is similar to that of *Chlamydia*. However, its pleomorphic shape, existence in phloem elements, and the possibility of artificial culture suggest that the likubin agent is closely related to the MLO so far described as phytopathogens and that it is not *Chlamydia*. Spiral forms and irregular constrictions in the likubin agent suggest a relationship between *Spiroplasma* and the likubin agent. In addition, the host range and mode of transmission of the likubin agent resemble those of *Spiroplasma citri*.

GRAFT INOCULATION EXPERIMENTS

The senior author made tissue-graft inoculations with budwood of likubin-infected Ponkan on indicator plants, Madam Vinous sweet orange, Sexton tangelo, and Cuban shaddock. The infected tangelo exhibited striking symptoms of chlorosis and stunting, while the infected sweet orange and shaddock showed milder symptoms. Eureka lemon and Mexican lime were then inoculated from the diseased tangelo. The infected Eureka lemon developed severe symptoms of seedling yellows; slight symptoms of stem pitting appeared on Mexican lime. This shows that the affected tangelo and Ponkan carried the agents of both likubin and tristeza. Based on these results, tangelo may not be an adequate indicator for likubin when the inoculum carries both agents; sweet orange seems to be preferable to tangelo.

TETRACYCLINE TREATMENT

Six infected tangelo and two sweet orange plants were treated with tetracycline in October 1970 by dipping their roots in 100 ppm chlortetracycline for 24 hours. About 15 months after treatment the infected plants showed remarkable or complete suppression of yellows or chlorotic symptoms, although the little-leaf

symptoms remained. Most untreated tangelo plants died within 3 years after inoculation. The reaction to tetracycline seems to be rather slow. However, citrus plants are usually slow to absorb nutrients and other chemicals through the roots. Absorption through the leaf surface is rather rapid but the effect does not last so long. The effect of tetracycline indicates that the cause of likubin is not virus, but a procaryotic organism.

DISCUSSION

Bové and Saglio (1974) pointed out that the greening agent has an envelope thicker than that of ordinary MLO or *Spiroplasma citri*, the causal agent of stubborn disease. Moll and Martin (1974) described the greening agent tentatively as an organism in a new group named "bacterium-like organism" (BLO), although they could not find reproduction by binary fission. Subsequently, much attention has been paid to the taxonomic positions of the greening agent and the likubin agent. For the moment, however, the authors consider that the likubin agent is also one of the mycoplasmalike organisms, although its membrane structure is different from that of other MLO. Moreover, as Goheen *et al.* (1973) and Nyland *et al.* (1973) indicated, pathogenic rickettsias of woody plants usually occur in the xylem elements. Further detailed investigations should be made to draw a definite conclusion concerning the likubin pathogen.

Another problem is the presence of virus particles in the likubin diseased plants. Chen *et al.* (1972) found filamentous particles of tristeza virus together with the likubin agent in the phloem elements. The authors, however, could not find tristeza virus particles in the affected tissues they examined. Instead, they demonstrated the presence of seedling yellows virus by means of graft inoculation (Martinez, 1972). Later, in continuing examinations of the inoculated indicators, the senior author found slight symptoms of stem pitting on Mexican lime inoculated with likubin-diseased tangelo and Cuban shaddock. This indicates that the original Ponkan carried MLO and a mild strain of tristeza virus.

The senior author is now working with the reaction of likubin-diseased plants to penicillin. It seems to be one of the key points in classifying the likubin agent, as Bowyer and Calavan (1974) and Windsor and Black (1973) suggested.

LITERATURE CITED

BOVÉ, J.M., and P. SAGLIO
1974. Stubborn and greening: a review, 1969-1972, p. 1-11, *In* L.G. Weathers and M. Cohen, (eds.), Proc. 6th Conf. Intern. Organization Citrus Virol., Univ. Calif. Div. Agr. Sci., Berkeley.

BOWYER, J.M., and E.C. CALAVAN
1974. Antibiotic sensitivity in vitro of the mycoplasmalike organism associated with citrus stubborn disease. Phytopathology 64: 346-49.

CHEN, M.H., T. MIYAKAWA, and C. MATSUI
1972. Simultaneous infections of citrus leaves with tristeza virus and mycoplasmalike organisms. Phytopathology 62: 663-66.

GOHEEN, A.C., G. NYLAND, and S.K. LOWE
1973. Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63: 341-45.

MARTINEZ, A.L.
1972. Combined effects of greening and seedling-yellows pathogens in citrus, p. 25-27. *In* W.C. Price, (ed.), Proc. 5th Conf. Intern. Organization Citrus Virol., Univ. Florida Press, Gainesville.

MOLL, J.N., and M.M. MARTIN
1974. Comparison of the organism causing greening disease with several plant pathogenic gram negative bacteria, rickettsia-like organisms, and mycoplasmalike organisms. INSERM 33: 89-96.

NYLAND, G., A.C. GOHEEN, S.K. LOWE, and H.C. KIRKPATRICK
1973. The ulstrastructure of a rickettsialike organism from a peach tree affected with phony disease. Phytopathology 63: 1275-78.

TANAKA, S.
1973. Tissue graft inoculation experiments with likubin and leaf-mottling onto the indicator plants. Ann. Phytopath. Soc. Japan 38: 211-12.

TANAKA, S. and Y. DOI
1974. Studies on mycoplasmalike organism suspected cause of citrus likubin and leaf-mottling. Bull. Faculty of Agriculture, Tamagawa University 14: 64-70.

WINDSOR, I.M., and L.M. BLACK
1973. Remission of symptoms of clover club leaf following treatment with penicillin. Phytopathology 63: 44-46.