Separation of a Mycoplasmalike Organism from the Likubin Complex in Citrus

CHIOU-HSIUNG HUANG, Plant Pathologist, Department of Plant Pathology, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan; MOJ-JIH CHEN, Professor of Plant Pathology, National Chung-hsing University, Taichung; and REN-JONG CHIU, Senior Specialist, Joint Commission on Rural Reconstruction, Taipei, Taiwan

ABSTRACT

HUANG, C.-H., M.-J. CHEN, and R.-J. CHIU. 1980. Separation of a mycoplasmalike organism from the likubin complex in citrus. Plant Disease 64:564-566.

Likubin mycoplasmalike organisms (MLOs) were separated from the likubin causal complex by using trifoliate orange seedlings to filter out tristeza virus. When Ponkan budsticks carrying the likubin MLO were grafted onto lime rootstocks, characteristic symptoms of likubin developed in 3–4 mo. The trees did not carry tristeza, according to the Mexican lime index test. Leaves from diseased plants contained MLOs in sieve tubes but not tristeza virus. Likubin MLOs and the likubin causal complex induced similar symptoms in tristeza-tolerant scion-stock combination citrus plants but dissimilar symptoms in tristeza-susceptible plants. Presence of tristeza virus seemed to suppress the yellowing symptom of MLOs, but the disease was more severe in plants infected with the causal complex than in plants infected with likubin MLO alone.

Citrus likubin is the most serious disease of citrus trees in Taiwan and can affect almost all major kinds of citrus plants. Symptoms consist of yellowing and corking of veins, mesophyll chlorosis, and unseasonal fall of leaves. Infected trees die 2–4 yr after onset of symptoms.

The etiology of the disease was not fully determined until after 1970 (3,14). The disease was once thought to result from unfavorable soil conditions, but studies showed that it was probably caused by tristeza or a closely related virus (13). Tristeza virus was found in healthy appearing citrus trees (7,15), suggesting that the citrus likubin causal complex must include an additional component (15).

In 1967, Doi et al (6) found mycoplasmalike organisms (MLOs) consistently associated with mulberry dwarf, aster yellows, and potato and paulownia witches'-brooms. MLOs or similar agents were also associated with citrus stubborn in California (10) and citrus greening in South Africa (2). Electron microscopic studies in the early 1970s showed MLOs in citrus affected by likubin (3,14). Because tristeza was also detected in healthy appearing trees, likubin is generally believed to be a mycoplasma disease.

Ponkan, Tankan, and orange trees with likubin but free of tristeza virus are difficult to obtain because of the sensitivity of MLOs to heat treatment (8,14) and chemotherapy (5,14). Until likubin MLOs free from tristeza virus can be obtained, the citrus likubin syndrome cannot be definitely attributed to a component in the causal complex.

We attempted to separate the MLO from likubin complex and to compare the reactions of citrus plants infected by MLO alone and by the complex. Preliminary reports of some of these studies have been published (9).

Contribution 893 from the Taiwan Agricultural Research Institute. This study was supported by the Joint Commission on Rural Reconstruction under projects 76-A-1.3-A-2506 and 77-ARDP-5.1 A-224.

0191-2917/80/06056403/$03.00/0

MATERIALS AND METHODS

Ponkan and Mexican lime seedlings were used as indicator plants for citrus likubin and tristeza virus, respectively. The source of likubin complex was a 10-yr-old Ponkan tree from an orchard in Teinleng, Taichung. It was free of viruses such as psorosis, concave gum, and exocortis, but it produced symptoms of likubin in Ponkan and symptoms of tristeza in Mexican lime seedlings. Tristeza virus was isolated from a symptomless Ponkan tree in Tungshih, Taichung.

Separation of MLO. *Citrus sunki*, Troyer citrange (*Poncirus trifoliata* × *C. sinensis*), *P. trifoliata*, *Limonia acidissima*, and *Murraya paniculata* were used as filter plants. Four seedlings of each species were bud-inoculated with likubin complex, and 8 mo later, plants with leaf yellowing or other symptoms were grafted onto Ponkan plants on Mexican lime rootstock.

Reactions of these plants served as the first indication of separation of likubin MLOs from the likubin complex. Isolates thought to be free of virus were reindexed on Ponkan and Mexican lime seedlings and diseased leaves examined with an electron microscope.

Electron microscopy. Diseased leaves were taken from Ponkan seedlings that had been inoculated 4–6 mo previously with what we presumed was likubin MLOs alone. Lateral vein tissues from leaves with yellowing or yellowing and corking were prepared for thin sections as described previously (4).

Reactions of citrus plants. Young scions of Ponkan infected with likubin MLO, likubin causal complex, and tristeza virus singly or in combination were grafted onto tristeza-tolerant rootstocks of Sunki and Rangpur lime. The three agents also were bud-inoculated to the tristeza-susceptible grapefruit and lemon seedlings and to Mexican or Rangpur lime. Paiyau Pummelo was also grafted onto Rangpur lime.

RESULTS

Separation of MLO. Tristeza virus alone or MLO and tristeza infected Sunki and Troyer citrange, but MLO was not isolated from the causal complex. Tristeza virus also infected *L. acidissima* seedlings. Neither tristeza nor MLO passed through *M. paniculata*, either because it was an unsuitable host or more probably because of poor compatibility between *M. paniculata* and citrus tissues,

Table 1. Growth of citrus plants[a] inoculated with tristeza virus, likubin mycoplasmalike organisms (MLO), and likubin causal complex

Citrus plant	First flush (cm)[b]			
	Tristeza virus	Likubin complex	Likubin MLO	Control
Paiyau Pummelo × Mexican lime	4.2	1.3	9.3	12.1
Paiyau Pummelo × Rangpur lime	6.8	7.2	18.7	18.9
Grapefruit seedling	6.3	7.3	15.4	17.2
Lemon seedling	15.1	12.2	20.1	22.5

[a] Observed 4 mo after graft-inoculation.
[b] Average of three inoculated plants for each treatment.

since the scions survived less than 1–2 mo.

Both MLO and tristeza appeared to be capable of infecting trifoliate orange, but some tissues carried MLO alone and we were able to obtain MLO free of tristeza by sampling such tissues.

Electron microscopy. MLOs were found in Ponkan plants that were free of tristeza virus. MLOs were more abundant in old leaves with vein corking than in young leaves with yellowing or chlorosis. We could not find tristeza virus in thin sections of leaf samples, suggesting that MLO was separated from tristeza virus by passing through trifoliate orange filter plants.

Reactions of citrus plants. Likubin MLOs alone and the likubin causal complex induced yellowing and vein corking in new leaf growth 3–4 mo after Ponkan scions were grafted onto Sunki and Rangpur lime rootstocks. Symptom expression did not differ between the scion-stock combination plants.

During the 1-yr observation, the Ponkan budwood infected only with tristeza virus remained free of symptoms on Sunki and Rangpur lime stocks. The tristeza-susceptible grapefruit and lemon seedlings and also the scion-stock combinations of Paiyau Pummelo on Mexican lime and Rangpur lime were considerably dwarfed (Table 1) and had small leaves 1–2 mo after inoculation with the likubin complex and with the tristeza virus alone, but differences were noticeable (Fig. 1). Infection with tristeza induced milder symptoms and no leaf yellowing.

Inoculation with likubin MLOs alone did not induce conspicuous symptoms in early stages in tristeza-susceptible plants or scion-stock combinations. Healthy controls and plants inoculated with likubin MLOs did not show significant differences in growth, at least for the first 2 mo, but inoculated plants had leaf yellowing later. Leaf yellowing seemed to be somewhat suppressed in plants infected with both MLO and tristeza virus, but the entire syndrome was more severe than that caused by likubin MLO alone in late stages.

DISCUSSION

The MLO suspected of causing likubin in Taiwan resembles the organism associated with citrus greening in South Africa (2) and leaf mottling disease in the Philippines (1,2). In the latter, the psyllaborne MLO often complexed with tristeza or seedling yellows virus in diseased plants, but the two components could be separated by vector transmission to susceptible citrus seedlings (12). MLO could also be separated from the causal complex by inoculation to trifoliate orange (11).

By using trifoliate orange as filter plants, we successfully isolated MLOs from likubin complex, specifically from tristeza virus. There was no apparent difference between MLO alone and likubin complex in tristeza-tolerant citrus plants such as Ponkan seedlings or Ponkan on Sunki and Rangpur lime rootstocks. In tristeza-susceptible plants, however, the likubin complex induced severe stunting, small leaves, and shortened internodes 1–2 mo after inoculation and later yellowing of leaves. Inoculation with tristeza virus induced similar symptoms, but without yellowing in the late stages. Similar results were

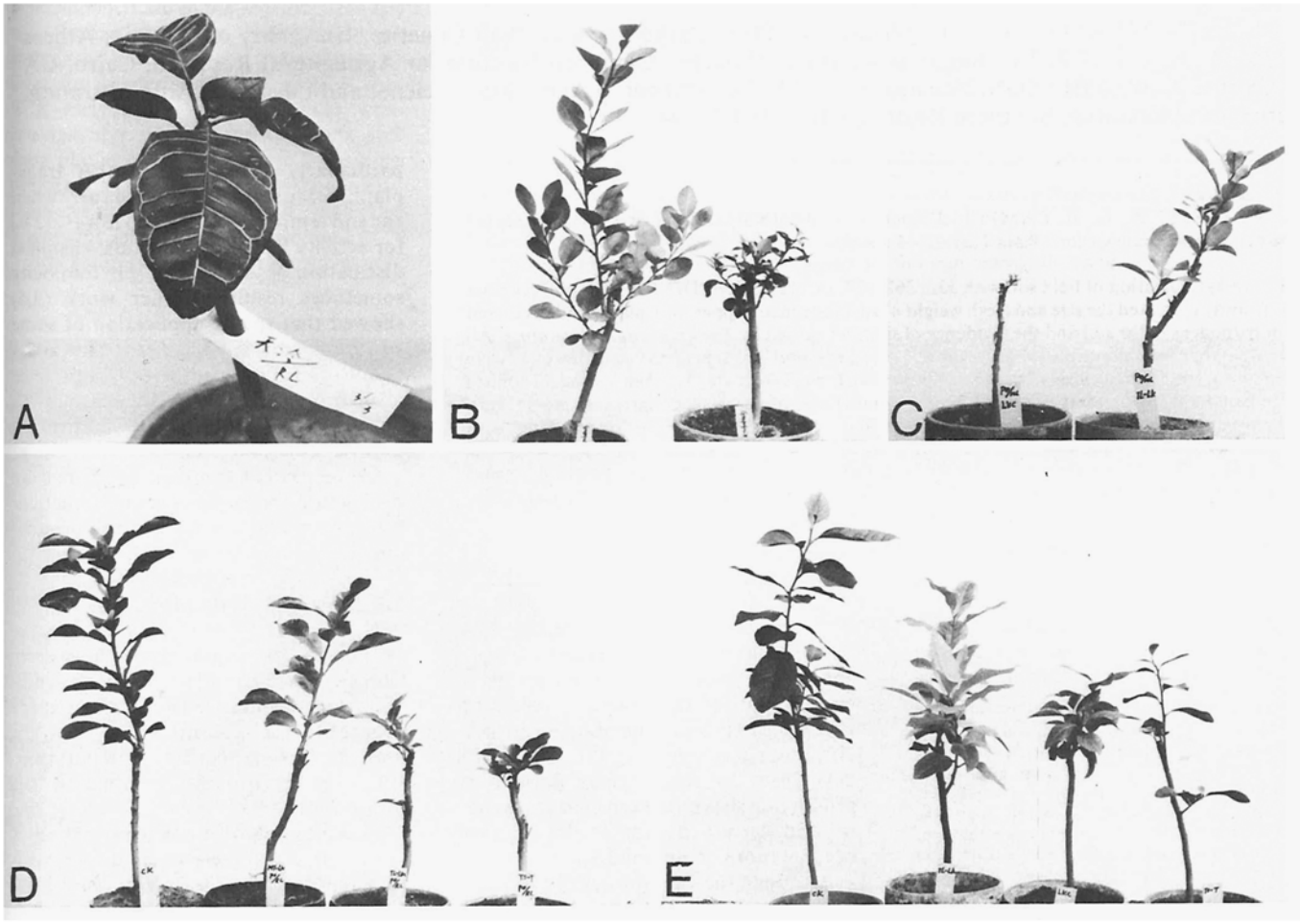

Fig. 1. Response of citrus plants to disease agents and combinations. **(A)** Yellowing and severe vein corking on scion of Ponkan inoculated with likubin mycoplasmalike organisms (MLOs), 6 mo after grafting on Rangpur lime rootstock. **(B)** Grapefruit seedlings inoculated with: likubin MLO (left) and likubin complex (right). **(C)** Paiyau Pummelo scions on Mexican lime rootstock inoculated with likubin MLO (right) and likubin complex (left). **(D)** Paiyau Pummelo scions on Rangpur lime rootstock (left to right): control and inoculation with likubin MLO, likubin complex, and tristeza virus. **(E)** Lemon seedlings (left to right): control and inoculation with likubin MLO, likubin complex, and tristeza virus. Photographs B, C, D, and E were taken 4 mo after inoculation.

reported for greening-seedling yellows complex (11).

LITERATURE CITED

1. ALTAMIRANO, D. M., C. I. GONZALES, and R. C. VINAS. 1976. Analysis of the devastation of leaf mottling (greening) disease of citrus and its control program in the Philippines. Pages 22-26 in: E. C. Calavan, ed. Proc. 7th Conf. Intern. Organ. Citrus Virol. University of California, Riverside.
2. BOVE, J. M., and P. SAGLIO. 1974. Stubborn and greening: A review, 1969-1972. Pages 1-11 in: L. G. Weathers and M. Cohen, eds. Proc. 6th Conf. Intern. Organ. Citrus Virol. University of California, Riverside.
3. CHEN, M. H., T. MIYAKAWA, and C. MATSUI. 1971. Mycoplasma-like bodies associated with likubin-diseased Ponkan citrus. Phytopathology 61:598.
4. CHEN, M. J., and N. J. KO. 1978. Cytological changes induced by transitory yellowing virus in infected rice plants. Plant Prot. Bull. (Taiwan) 20:46-55.
5. CHIU, R. J., M. Y. TSAI, and C. HS. HUANG. 1978. Tree transfusion with tetracyclines as a measure for citrus likubin control. Pages 91-103 in: Proc. Symp. Nov. 30-Dec. 3, 1977, Tokyo, Japan. FFTC Book Series 13. Food and Fertilizer Technology Center for the Asian and Pacific and Pacific Region. Taipei, Taiwan.
6. DOI, Y., M. TERANAKA, K. YORA, and H. ASUYAMA. 1967. Mycoplasma or PLT group like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows or paulownia witches' broom. Ann. Phytopathol. Soc. Jpn. 33:259-266.
7. HUANG, C. HS. 1972. Studies on citrus virus diseases in Taiwan. J. Taiwan Agric. Res. 21(1):62-70.
8. HUANG, C. HS. 1978. Effect of hot-air treatment on likubin, tristeza virus and exocortis viroid diseases of citrus. J. Agric. Res. China 27(2):193-198.
9. HUANG, C. HS., R. J. CHIU, and M. J. CHEN. 1977. Isolation of the MLO component from citrus likubin complex. (Abstr.) Plant Prot. Bull. Taiwan 19:298.
10. IGWEGBE, E. C. K., and E. C. CALAVAN. 1970. Occurrence of mycoplasma-like bodies in phloem of stubborn infected citrus seedlings. Phytopathology 60:1525-1526.
11. MARTINEZ, A. L. 1972. Combined effects of greening and seedling-yellows pathogens in citrus. Pages 25-27 in: W. C. Price, ed. Proc. Conf. Int. Organ. Citrus Virol. 5th. University of Florida Press, Gainesville.
12. MARTINEZ, A. L., and J. M. WALLACE. 1969. Citrus greening disease in the Philippines. Pages 1427-1431 in: H. D. Chapman, ed. Proc. lst Int. Citrus Symp., Vol. 3. University of California, Riverside.
13. MATSUMOTO, T., M. C. WANG, and H. J. SU. 1961. Studies on likubin. Pages 121-125 in: W. C. Price, ed. Proc. Conf. Int. Organ. Citrus Virol. 2nd. University of Florida Press, Gainesville.
14. SU, H. J., and S. C. LEU. 1972. Study on the pathogen complex causing likubin of citrus in Taiwan. I. Nature of mycoplasma-like organism associated with the disease. Proc. Nat. Sci. Council 5:109-126.
15. SU, H. J., and T. MATSUMOTO. 1972. Further studies on the complex causing likubin of citrus in Taiwan. Pages 28-34 in: W. C. Price, ed. Proc. Conf. Int. Organ. Citrus Virol. 5th. University of Florida Press, Gainesville.