# Further Studies on the Complex Causing Likubin of Citrus in Taiwan

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LIKUBIN has been prevalent in Taiwan since 1958, attacking most of the commercial varieties such as ponkan, tankan, and sweet orange. Formerly thought to be due to unfavorable soil conditions, such as high moisture content and deficiency of essential nutrients, or to root rot, it was subsequently demonstrated to be caused by an infectious agent believed to be closely related to tristeza virus because it induced the tristeza reaction in West Indian lime and Eureka lemon and was transmitted by the tropical citrus aphid *Toxoptera citricidus* Kirk. (9, 10). The tristeza virus from likubin was, however, found to differ somewhat from that in other countries; it affected ponkan rooted on sunki, Cleopatra mandarin, and Troyer citrange rootstocks, which are reported to be resistant to tristeza virus in North and South America. *Aeglopsis chevalieri* also indexed positive with the virus. Moreover, positive reactions in lime seedlings were obtained by grafting them with scions from healthy looking ponkan and tankan trees on sunki rootstock. Because of these discrepancies, it was assumed that some unknown component, in addition to tristeza virus, was associated with likubin. This paper reports the results of investigations made to identify the unknown component and to clarify the nature of the complex.

## *Procedures and Results*

ELECTRON MICROSCOPY.—Leaf dip preparations derived from West Indian lime, ponkan, and Eureka lemon plants infected by means of aphids that had fed on a source of the likubin complex (LKC) were negatively stained and examined in an electron microscope. The preparations were seen to contain flexuous rods approximately 2000 nm long, corresponding in size and shape with those of tristeza virus described by Kitajima et al. (5, 6, 16) and by Price (12). It is concluded that tristeza virus definitely is present in likubin-infected tissue in Taiwan.

COMPARISON OF LKC WITH TRISTEZA VIRUS ISOLATED BY INSECT VECTORS OR FILTER PLANTS.—Indexing some healthy looking ponkan, tankan, and sweet orange trees growing on sunki rootstocks resulted in severe tristeza reactions in the lime index plants. From this result, it was concluded that one or more pathogens other than tristeza virus are involved in LKC. Experiments were then made to determine whether certain components of the LKC could be filtered out by certain citrus indicator plants and by aphid and psyllid vectors. In

a preliminary test, tristeza virus separated from LKC by filtration through Mexican lime or transmission by the tropical citrus aphid was less virulent in plants of ponkan and tankan rooted on sunki than the original LKC maintained in ponkan seedlings (Fig. 1,A,B). All inoculum sources derived from Eureka lemon, sunki, Rangpur lime, sweet orange, pon-

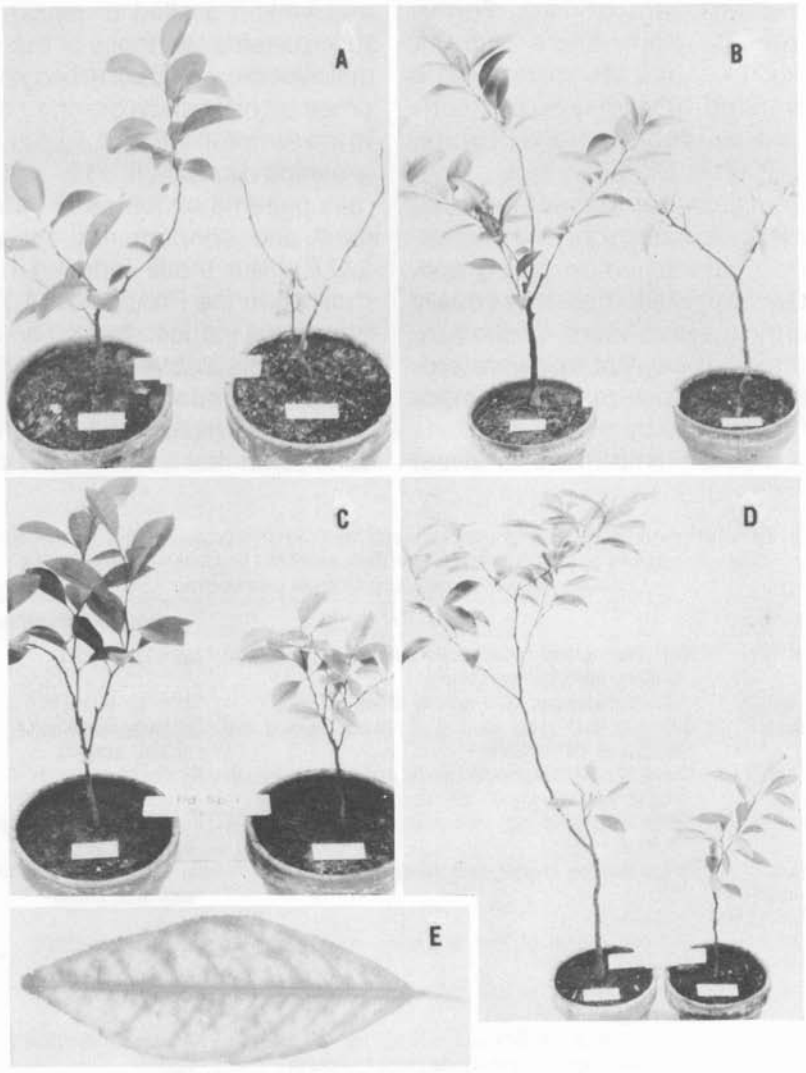


FIGURE 1. *Comparative response of citrus plants to likubin complex (right) and to tristeza virus (left). A Ponkan on sunki rootstock. B. Tankan on sunki. C. Szinkom seedlings. D. Calamandarin seedlings. E. Likubin-diseased leaf of Valencia sweet orange showing a chlorotic pattern with dark green spotting.*

kan, and tankan seedlings used as test filter plants produced severe symptoms in the test stock-scion combinations similar to those induced by the original LKC. Presumably these plants did not filter out the main component of LKC. Further experiments were made with the original LKC and its tristeza virus component. The results are summarized in Table 1 and Figures 1 and 2.

The tristeza component and LKC produced similar symptoms in Mexican lime, Eureka lemon, and grapefruit plants; they seemed to be equally virulent in these cultivars. On the contrary, no visible symptoms were produced by the tristeza virus component transmitted by means of aphids or by Mexican lime grafts to sweet orange, Rangpur lime, Etrog citron, ponkan, tankan, sunki, Szinkom, and calamandarin seedlings and to ponkan and tankan scions on sunki rootstock. The original LKC produced typical likubin symptoms in ponkan and tankan grafted on sunki, chlorotic patterns like those of nutritional deficiencies, and dark green spotting on leaves of Valencia orange—similar to the symptoms (Fig. 1,E) caused by greening disease (8, 11)—and chlorotic patterns on leaves of calamandarin and szinkom mandarin (Fig. 2,D,E)—like those induced by leaf mottling in the Philippines (13). The symptoms induced by LKC on sweet orange were also very similar to those of stubborn reported from the United States (1). These results lead to the conclusion that an unknown patho-

TABLE 1. COMPARATIVE RESPONSES OF VARIOUS INDICATOR PLANTS TO INOCULATION WITH THE LIKUBIN COMPLEX BY MEANS OF GRAFTS AND TRISTEZA VIRUS BY MEANS OF APHIDS OR BY MEANS OF GRAFTING WITH SCIONS FROM MEXICAN LIME PLANTS

| Indicator plant | Likubin complex (LKC) | Tristeza-virus component |
|---|---|---|
| Mexican lime | Vein clearing and corking; leaf flecking, cupping, and atrophy; stem pitting; stunting | Same as with LKC |
| Eureka lemon | Yellowing, chlorosis, and atrophy of leaf; stunting | Same as with LKC |
| Grapefruit | Chlorosis, dark green spotting on leaves; cupping and atrophy of leaf; stunting | Chlorosis, cupping, and atrophy of leaf, stunting |
| Sweet orange (Valencia) | Chlorotic pattern of zinc deficiency, and dark green spotting on leaf; atrophy of leaf; stunting | No visible symptom |
| Rangpur lime | Yellow mottling of leaf; stem pitting; phloroglucinol test negative | No visible external symptom except stem pitting |
| Etrog citron (Arizona 861 or USDCS 60-13) | Yellow mottling of leaf; stem pitting | No visible external symptom except stem pitting |
| Szinkom mandarin | Chlorotic pattern of zinc deficiency, atrophy of leaf; stunting | No visible symptom |
| Calamandarin | Chlorotic pattern of zinc deficiency, atrophy of leaf; stunting | No visible symptom |
| Ponkan/Sunki | Vein yellowing, swelling and corking; leaf yellowing or chlorosis; rigid; premature defoliation; dieback | No striking symptom; loss of vigor |
| Tankan/Sunki | Symptoms similar to those in ponkan/sunki, in addition to mild stem pitting | No visible symptom |
| Sunki | Leaf yellowing with deficiency patterns, vein swelling, and corking | No visible symptom |

gen, in addition to tristeza virus, is involved in likubin disease and that this unknown component may be the main pathogen of this disease. Evidently it was filtered out by passage of LKC through the aphid vectors and by passage through Mexican lime seedlings.

IDENTIFICATION OF THE UNKNOWN COMPONENT OF LKC.—Plants infected with LKC were indexed into seedlings of cultivars regarded as indicator

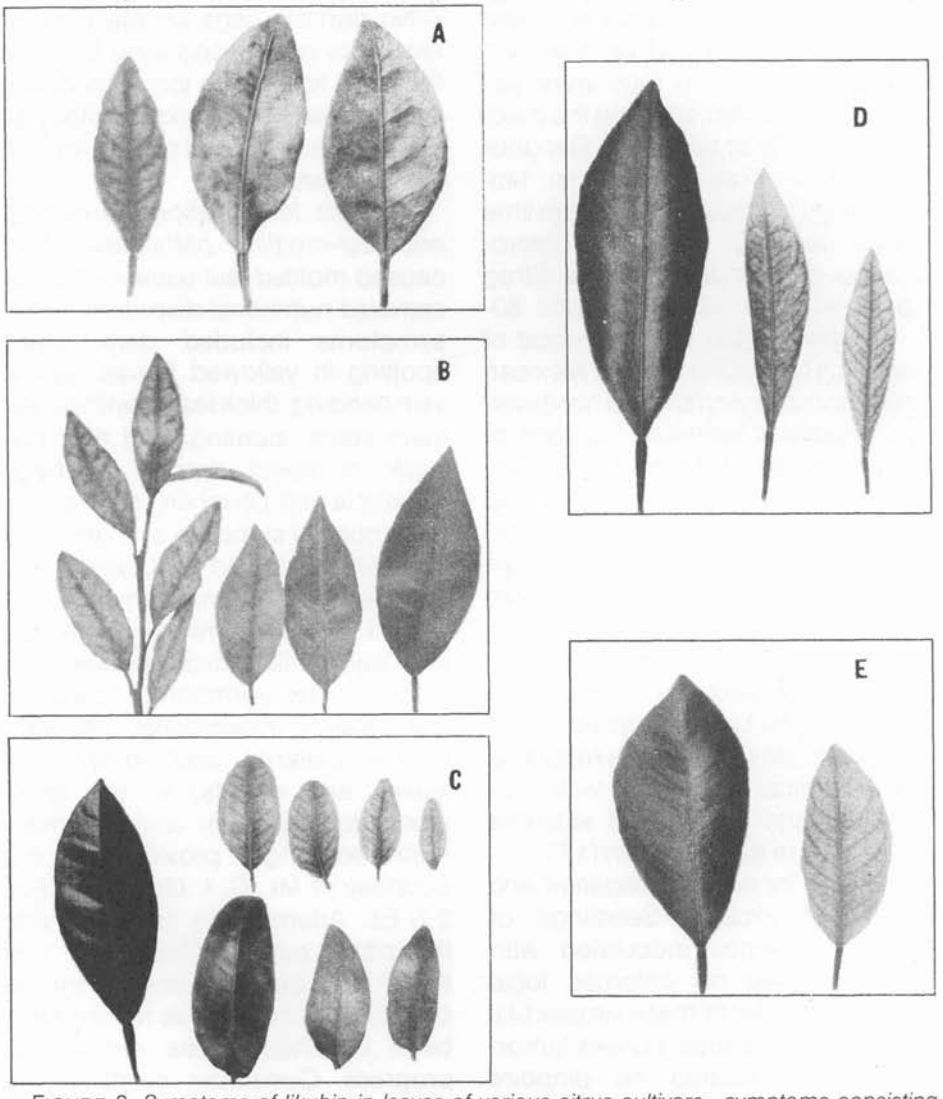


FIGURE 2. *Symptoms of likubin in leaves of various citrus cultivars—symptoms consisting of prominent and corky veins, chlorotic patterns resembling those of nutritional deficiencies, and dark green spotting. A. Ponkan. B. Tankan. C. Sunki. D. Szinkom mandarin. E. Calamandarin.*

plants for certain citrus viruses, as follows.

*A. Tests for exocortis virus.*—Ponkan, tankan, and sweet orange scions carrying LKC were grown on rootstocks of trifoliate orange and Rangpur lime. No bark scaling or yellow blotching was noticed on the rootstocks although the tops were yellowed and stunted. Staining the medullary ray cells of bark from Rangpur lime, trifoliate orange, ponkan, tankan, sweet orange, and Mexican lime plants carrying LKC with phloroglucinol gave negative results. Etrog citron (Arizona 861 and USDCS 60-13) plants grafted with budwood of ponkan, Rangpur lime, and Mexican lime plants carrying LKC did not develop epinasty of leaves or cracking of the underside of the midveins. The citron plants did, however, develop yellow mottled leaves and stem pitting. It is concluded that the unknown component of LKC is not exocortis virus.

*B. Tests for satsuma dwarf virus.*—Seedlings of blackeye cowpea and white sesame, kindly supplied by Dr. S. Yamada, gave a negative response to mechanical inoculation with LKC, indicating the absence of satsuma dwarf virus in the inoculum (17).

*C. Tests for citrus-variegation and crinkly-leaf viruses.*—Seedlings of blackeye cowpea inoculated with LKC developed no chlorotic local lesions indicative of these viruses (4). Leaves of inoculated Eureka lemon seedlings developed no pinpoint spotting.

*D. Tests for concave-gum and psorosis viruses.*—Sweet orange seedlings inoculated with LKC did not develop the young-leaf symptoms (vein flecking and zonate oakleaf patterns) of psorosis A and concave gum.

*E. Tests for cachexia-xyloporosis.*—No gummy pegs on the bark or small pits in the wood were found in Rangpur lime stock topworked with likubin-diseased ponkan, although some groove-shaped pits did form in the stock wood.

*F. Tests for stubborn, greening, and leaf-mottling pathogens.*—LKC caused mottled-leaf patterns that resembled nutritional disorders. Other symptoms included dark green spotting in yellowed leaves, yellow vein banding, thick leaves with prominent veins, stunting, and twig dieback in sweet orange seedlings (Valencia and Leuchen) and resembled those of stubborn and greening diseases described by Calavan and Christiansen (2) and Schwarz (14) (Fig. 1,E). The similarity of likubin and leaf-mottling diseases was also noted in the symptoms (chlorotic leaf patterns resembling zinc-deficiency patterns and atrophy of leaves and shoots) in the graft-inoculated Szinkom and calamandarin seedlings, provided by the courtesy of Mr. C. I. Gonzales (Fig. 2,D,E). Attempts to transmit with the citrus psyllid (*Diaphorina citri* Kuw.) have been made since the fall of 1968, but no positive results have been obtained. Trials are still in progress. Cucumber seedlings inoculated with the likubin pathogen did not develop symptoms. Chromatographic analyses of albedo and

bark extracts from likubin-diseased ponkan, tankan, and sweet orange plants were made by the methods described by Schwarz (15) for detecting the fluorescent marker substance considered to be specific for the greening disease. Bright violet-blue spots were barely visible in the chromatic profile at Rf 0.1 under UV light.

## *Discussion and Conclusions*

In previous reports (9, 10) it was suggested that likubin is caused by a virus or viruses closely related to that of tristeza. It is now apparent that an unknown component is associated with this disease and that this unknown component may be the main component of the causal complex. Tristeza virus is present even in healthy looking trees in Taiwan. Moreover, tristeza virus isolates from LKC did not produce likubin symptoms in commercial stock-scion combinations whereas grafts from likubin-affected trees caused typical likubin symptoms.

Latent infection by tristeza virus is widespread in Taiwan in ponkan, tankan, Leuchen, and Valencia sweet orange trees on sunki rootstock, but these combinations are tolerant of the virus. The effect of tristeza virus on the development of likubin is still undetermined because isolates of the unknown component free of tristeza virus are still unavailable. Nevertheless, it is apparent that tristeza virus does not add materially to the severity of the disease because in a preliminary experiment inoculum buds from trifoliate orange plants—which are immune from tristeza virus—graft-inoculated with LKC, recently induced likubin symptoms as severe as those caused by the original LKC

Calavan (1) suggested that likubin in Taiwan and yellow shoot in mainland China (3, 7) are similar to greening and stubborn in many respects. In view of this similarity, it is likely that the main component of LKC is a pathogen closely related to those that cause stubborn, greening, and leaf-mottling diseases. Nevertheless, chromatographic analyses of the albedo and the bark of likubin-affected trees have failed to give a positive fluorescent test, and attempts to transmit the disease by means of the citrus psylla have been negative. Thus, it is too early to conclude that the likubin pathogen is related to the greening pathogen or to the stubborn pathogen.

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