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Studies on Likubin

LIKUBIN was formerly considered as being due to unfavorable soil conditions such as high moisture or deficiency in essential nutriments. However, the spread of the disease could not be checked by improvement of soil conditions but tended to increase year by year. Accordingly, at the request of the authorities concerned, we undertook a thorough study of this disease 3 years ago.

Although the symptoms vary according to environmental conditions and varieties of citrus trees, the general appearance is a decline in vigor, yellowing or mottling of leaves (Fig. 1, A), premature defoliation, dieback of small twigs, formation of multiple abnormal flowers, decay of feeder rootlets and lateral roots, and wilting of leaves, followed by death of the entire plants.

On the assumption that Likubin may be of a virus nature, extensive studies were made by grafting diseased Ponkan and Tankan trees collected from almost all the citrus-growing districts onto Sunki-seedlings, which are used principally as rootstocks in Taiwan. In addition, some trials of consecutive grafting were made by inarching simultaneously several potted Sunki-seedlings. Transmission studies were also made by means of insect vectors (*Toxoptera citricidus* = *Aphis citricidus*).

In parallel with the aforementioned transmission studies, we also directed attention to identification of the virus. From the symptomatological studies, this disease seems to be very much like quick decline or tristeza, which is prevalent in North and South America. Therefore, the first step was taken by bud- or leaf-grafting to Mexican lime seedlings, the seeds of which were provided by courtesy of Dr. J. M. Wallace, and it was then made clear that most of the diseases hitherto considered

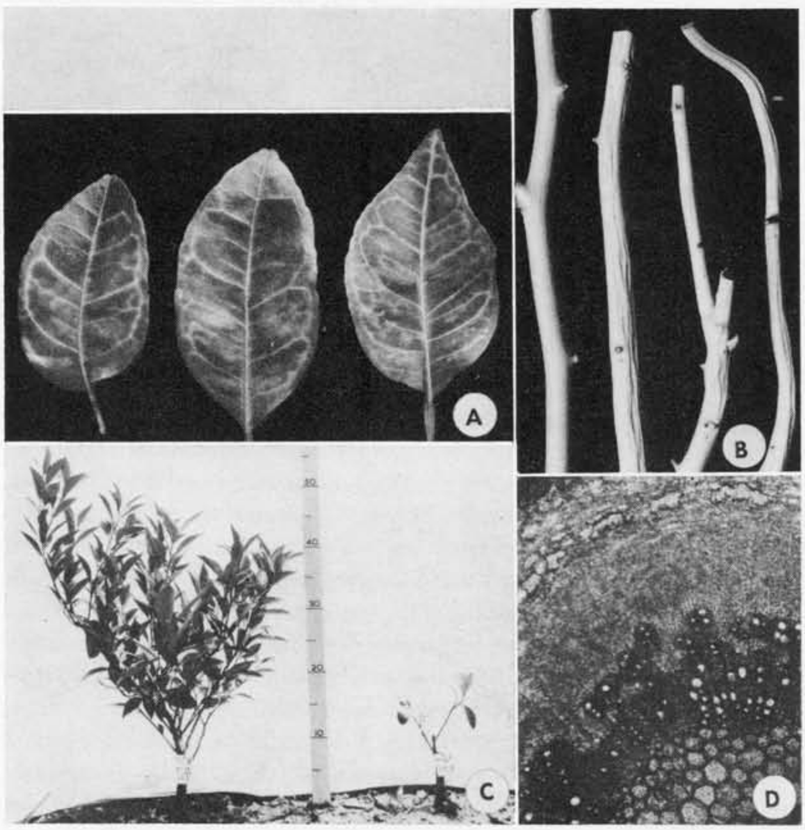

FIGURE 1. *A. Leaf symptoms exhibited by a tristeza-infected Ponkan tree, showing yellowing of veins and mesophyll-tissues surrounding the former. B. Stem pitting produced on the stem of Aeglopsis chevalieri infected by tristeza (left: normal). C. Tankan on Sunki rootstock inoculated with UO 1 after prior inoculation with YMS 15 (left) and Tankan on Sunki rootstock inoculated with YMS 15 alone (right). (Photographed 15 months after the first inoculation.) D. Transverse section of a tristeza-infected Mexican lime stem, showing the intrusion of phloem elements into the xylem portion.*

as nonparasitic Likubin should be included in the tristeza group.

It has recently been pointed out by Knorr (2) that quick decline occurring in U.S.A. is different from tristeza occurring in South America in reaction of *Aeglopsis chevalieri*. Therefore, some experiments were undertaken with seedlings of this species, seeds of which were also kindly supplied by Dr. Wallace. The test made it clear that at least some of our tristeza is more closely related to that of South America than to that of North America, since the test plants exhibited vein clearing, cupping of leaves, and stem pitting two months after inoculation (Fig. 1,B).

In our preliminary studies, Ponkan and Tankan on Sunki rootstock inoculated with UO 1 virus after prior inoculation with YMS 15 virus and vice versa revealed no symptoms even after 15 months, while both plants inoculated with either UO 1 or YMS 15 alone were severely diseased. On the other hand, the same kinds of plants cross-inoculated with either UO 1 and UO 14 or YMS 15 and UO 14 were not appreciably protected, but exhibited typical symptoms of tristeza within one year (Fig. 1,C). Thus, it seems that there exists an interference between UO 1 and YMS 15, but not between either UO 1 and UO 14 or YMS 15 and UO 14. No interpretations of the mechanism of this interference can be made at present, since it has not yet been clarified whether these strains are simple or consist of more than one virus. In this connection, some virus strains, e.g., YMS 15, UO 1, UO 14 and T 2, gave a reaction on Eureka lemon similar to reaction 2 of Fraser's test (1).

Studies on the reaction of stock-scion combinations to the disease were carried out by use of the following rootstocks: trifoliata, Cleopatra, Troyer citrange, *Citrus junos, Citrus Tachinaba, Citrus tangerin*. The Ponkan tops on Cleopatra proved to be rather susceptible to our virus despite the fact that the latter plant is considered tolerant of tristeza in North and South America and satisfactory as a rootstock for commercial varieties of sweet orange. On the other hand, Ponkan tops on trifoliata rootstock appear to be more tolerant than any of the others tested. This result is apparently in accord with the fact that the rootstock of trifoliata orange is classed as tolerant in the aforementioned countries.

By macrochemical and microchemical tests, starch was absent from roots of Sunki-stocks as well as from shoots of the virus-affected Ponkan on the former, while in the corresponding parts of the control plants starch had accumulated. On the other hand, starch was present in diseased leaves in higher amounts except at the advanced stage of infection, at which time the leaves became more or less rigid and pale yellow rather than greenish yellow. It is conjectured that more starch in the affected leaves may be due to an impediment in the transportation of carbohydrates on account of phloem necrosis occurring in the foliar vascular elements, and that the reduction or disappearance of starch in the pale yellowish leaves or those on the verge of death is brought about by utilization of starch under a limited supply of photosynthetic products.

Amylase activity in the diseased Ponkan and Tankan leaves was generally higher, with some exceptions, than in healthy leaves of corresponding age. These results are at variance with the finding of Trippi and Mesias (4) that the activity of amylase was less in tristeza-affected citrus plants in Argentina. It is to be noted, however, that the data obtained from the experiment with Mexican lime were not clear-cut, since in some cases amylase activity appeared to be slightly weaker in the diseased leaves than in the normal-appearing ones borne on the same plant. It seems likely that amylase activity may differ according to the varieties tested as well as their leaf conditions, and that the abnormal accumulation of starch in the Ponkan and Tankan leaves may strengthen the activity of amylase owing to the adaptive nature of the latter.

It was demonstrated by paper chromatography that in the virus-affected Mexican lime leaves glucose and fructose were somewhat more abundant than in those of the healthy ones, while sucrose remained constant. Paper chromatographic techniques were also applied for the demonstration of nonvolatile organic acids in virus-affected leaves. Although the study is preliminary and still in progress, it seems that citric, malic, and succinic acids are rather remarkably reduced in both the virus-affected Tankan and Mexican lime leaves, while oxalic acid was slightly increased.

As has already been pointed out by Trippi and Mesias (4), chlorophyll A and B were less in the virus-affected Tankan leaves, while carotenoids were more abundant in the diseased.

Some histological studies have been made on the leaf and stem of Mexican lime inoculated by means of bud-grafting. These experiments had been mostly done before Schneider's paper (3) came to hand. Our findings that bast fiber cells in the leaf tissues of cleared veins are almost absent and that the phloem elements plunge into the xylem portion appear to be in harmony with Schneider's (Fig. 1,D), though as to the causation of vein clearing and chromatic cells described by him no comment can be made at present, since our experiment has not yet been extended so far.

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