Leaf Mottling—a Serious Virus Disease of Citrus in the Philippines

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SEVERAL HUNDRED THOUSAND citrus trees have died or are declining in Batangas province in the Philippines (5, 6). The causal disease was first noted by government technicians of the Lipa Experiment Station as early as 1957, and since then the number of affected trees has been increasing constantly. The disorder was at first thought to result from microelement deficiencies, but affected trees failed to respond to corrective sprays. The spread of the disease from orchard to orchard suggests that it is caused by a virus. Presently, the disease occurs within a radius of 80 to 100 kilometers from Lipa City in Luzon Island, and in one orchard at Calapan, Oriental Mindoro. The name leaf mottling or yellow mottle leaf was given to the disease because the most striking symptom is the mottled leaves of affected trees.

SYMPTOMS.—Diseased trees develop mottled leaves and dieback of young branches; they become uneconomical within a few years. The leaf mottling is similar to that caused by deficiencies of zinc, manganese, iron, boron, and certain other microelements. Variations in mottle patterns were observed in different varieties of citrus. For example, Szinkom mandarin trees (*Citrus reticulata* Blanco) frequently develop zinc deficiency patterns with the leaves reduced in size and growing in an upright position (Fig. 1,A). Leaves of Szwuikom and Tankan mandarins tend to roll, become leathery, and develop a dull yellowish green color suggesting boron deficiency.

Premature leaf drop and dieback of twigs of the more affected branches are other symptoms associated with the disease. Fruits of affected trees are undersized and often fall before maturing. Browning and death of the peduncle and stem end portion of the fruit often occurs on more severely affected branches of Szinkom mandarin trees. This symptom is less common on Ladu mandarin, although many Ladu fruits become discolored and develop hardening of the albedo at the stem end. Underdeveloped fruits often color irregularly starting at the stem end (Fig. 1,C).

The disease commonly starts in one branch and progressively involves the entire tree. In uprooted trees, the root system was observed to be poorly developed. Trees in several stages of decline commonly occur in

the same orchard. The disease occurs in trees of different ages, but most commonly appears in trees six or seven years of age, after the first heavy crop.

The iodine test indicated an accumulation of starch in the rootstock immediately below the bud-union in many leaf-mottled trees of Szinkom, Ladu, Szwuikom, and Taikat mandarin on calamandarin (*C. reticulata*

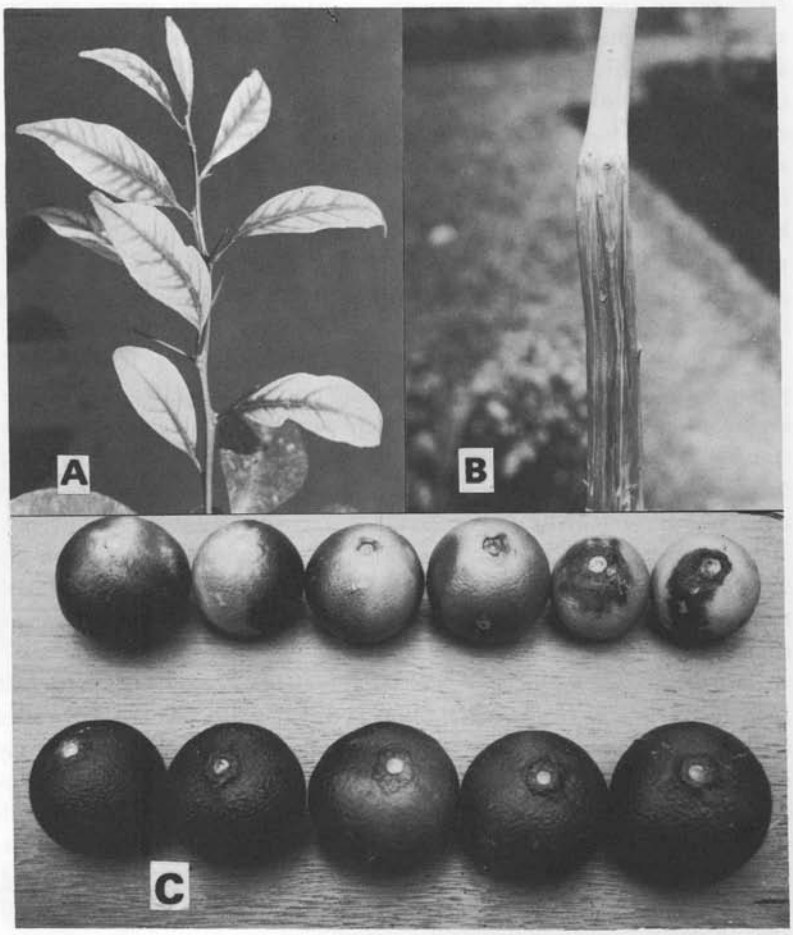

FIGURE 1. *Leaf-mottling disease symptoms. A. Mottled leaves of Szinkom mandarin. B. Trunk of Szinkom mandarin on calamandarin stock treated with iodine to show accumulation of starch in the stock. C. Fruits of diseased Szinkom mandarin (upper row) and healthy mandarin (bottom row).*

Blanco) rootstock (Fig. 1,B). The starch content is higher in mottled leaves than it is in healthy ones, and the midrib diameter is greater, sometimes twice that of leaves from adjacent healthy branches.

Methods and Results

TRANSMISSION EXPERIMENTS WITH BUD INOCULUM.—Eight transmission experiments were conducted, under both screenhouse and field conditions. In the first experiment, buds of nucellar Szinkom mandarin were grafted on young seedlings of the following citrus varieties: Rangpur lime [*C. aurantifolia* (Christm.) Swing.]; Mazoe Rough lemon [*C. limon* (L.) Burm. f.]; calamandarin, Cleopatra, Batangas, Szinkom, Ransas, Ladu mandarins (*C. reticulata* Blanco), and King mandarin (*C. nobilis* Lour.); Troyer, Morton, and Carrizo citranges [*C. sinensis* (L.) Osb. x *Poncirus trifoliata* Raf.]; Orlando tangelo (*C. reticulata* Blanco x *C. paradisi* Macf.); Hinkley sweet orange [*C. sinensis* (L.) Osb.]; sour orange (*C. aurantium* L.); and *C. macrophylla* Wester. Three plants on each rootstock were inoculated with blind buds from 2 different diseased Szinkom mandarin trees and from 1 leaf-mottled Gayonan mandarin tree. Three non-inoculated plants on each rootstock were left as controls. Leaf mottling and stunted growth developed in most trees irrespective of the rootstock. Inoculated plants on the sour orange rootstock died, apparently due to the presence of tristeza in the inoculum.

In the second experiment, young seedlings of calamandarin, Ladu, Szinkom, and Szwuikom mandarins, Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.], Mazoe Rough lemon, Rangpur lime, trifoliate orange, Comprida citron (*C. medica* L.), sour orange, sweet orange, and *C. macrophylla* Wester were inoculated with buds taken from a diseased Gayonan mandarin tree. Three seedlings of each variety were inoculated and 3 others were maintained as controls. Twenty days after inoculation all seedlings were pruned back. After 5 months, the inoculated Szinkom and Szwuikom mandarin seedlings showed severe leaf mottling and stunting (Fig. 2,A). Inoculated calamandarin seedlings were severely stunted and developed small, yellowish, cupped leaves. Inoculated Rough lemon seedlings developed small leaves with irregular chlorotic areas, and the new sprouts were stunted (Fig. 2,B). These symptoms, however, did not persist in the succeeding flushes 10 months after inoculation. Comprida citron and *C. macrophylla* seedlings developed yellowish new leaves. All of the mandarin seedlings tested exhibited stunted growth with characteristic mottling of the leaves. Sour orange and sweet orange showed yellow mottling, but were not markedly stunted. Rangpur lime seedlings were

only slightly stunted. No significant difference was observed between inoculated and non-inoculated trifoliate orange seedlings.

In a third experiment, bud inoculum was taken from 2-year-old field-grown seedlings of calamandarin and Eureka lemon that showed severe yellow mottling of leaves. Budwood from 4 diseased seedlings of calamandarin and 2 of Eureka were used to inoculate Key lime and sweet orange seedlings. Only 1 of the diseased seedlings was found to be carry-

FIGURE 2. *Seedlings inoculated with leaf-mottling virus and healthy controls. A. Szinkom mandarin. B. Florida Rough lemon.*

ing tristeza virus as revealed by the Key lime test plants. The sweet orange test plants inoculated with buds from the other 3 calamandarin seedlings, and from the Eureka lemon seedlings, developed small yellowish green leaves and remained stunted. The Key lime seedlings showed only mild yellowish symptoms in the new leaves.

Other experiments were conducted mainly to determine the susceptibility of different citrus varieties to the leaf-mottling virus. Thirty different varieties were tested. Mandarin varieties were the most severely affected. Ponkan, Szwuikom, Tankan, and Szinkom were the most susceptible. Rough lemon proved to be very sensitive at the early stage after infection, whereas Rangpur lime was moderately affected. Sour orange and

sweet orange are affected, but not severely. Trifoliate orange is apparently tolerant. Under Philippine conditions, Ponkan and Szwuikom mandarins are considered good indicator plants for the leaf-mottling virus.

INSECT TRANSMISSION.—The aphid *Toxoptera citricida* Kirk. was collected from diseased trees and was used to infect healthy test plants of Szinkom on calamandarin rootstock. This insect successfully transmitted the tristeza virus, but failed to transmit the agent responsible for leaf-mottling disease. The presence of the tristeza virus in the inoculated test plants was detected through the Key lime test.

Trials using the citrus psylla, *Diaphorina citri* Kuway, were started, and preliminary results indicate that this insect is the vector of the disease. Extensive experiments on insect transmission are being carried out in collaboration with the Entomology Section of the Lipa Experiment Station. Although details of the work are not presented in this report, C. S. Celino *et al.* (9) have demonstrated that *D. citri* Kuway is the vector. A large population of this insect is present in the Batangas area, but it was seldom observed in other areas of the Philippines.

CONTROL EXPERIMENTS.—Several experiments on control methods were commenced in commercial orchards. Inarching experiments, application of microelements coupled with lime application to the soil, pruning and topworking trials, and attempts to induce the scion to produce roots of its own are some of the methods tried. Certain experiments were based on the assumption that leaf-mottling disease is a scion-rootstock problem, similar to tristeza, and they were undertaken before the true nature of the disease was discovered. This fact explains the disappointing response of leaf mottle-affected trees to practically all treatments. In one experiment, branches of diseased Szinkom mandarin trees were inarched with potted seedlings of seven different varieties. All inarched branches failed to recover. The seedling rootstocks also developed the disease, with the exception of those of trifoliate, which grew normally.

Discussion and Conclusions

Transmission experiments have proved the virus nature of the leaf-mottling disease. Field observations clearly indicate that the virus has a vector other than man, and preliminary results obtained by the authors of this paper indicated that the vector is the citrus psylla *D. citri* Kuway. This fact was confirmed in later studies (9). Insect transmission experiments have shown that the aphid *T. citricida* Kirk. does not transmit the leaf-mottling virus.

The disease has certain features resembling tristeza. However, the

failure of *T. citricida* Kirk. to transmit the virus, the difference in host reaction, and the accumulation of starch in the rootstock portion of the trees all indicate that the leaf-mottling virus is not a strain of tristeza (5, 6, 9). The extent to which tristeza virus influences the symptoms of leaf-mottling disease remains to be determined.

The host range and the symptoms of leaf-mottling disease are similar to those of certain virus diseases recently reported to be affecting citrus trees in other countries of Southeast Asia. For example, a virus disease causing chlorosis on mandarins and other citrus plants was recently reported from Java (8) and is considered responsible for the decline of hundreds of thousands of citrus trees in that country. A destructive disease named yellow shoot is seriously affecting the citrus of southern China (1, 3). The symptoms of Likubin disease in Taiwan are very similar to those of leaf mottling in the Philippines. According to Dr. Su (personal communication), nearly one-third of the citrus trees in Taiwan show Likubin symptoms. Symptoms of leaf-mottling disease closely resemble those reported for greening and reticulata yellows disease of South Africa (4, 7). The citrus decline in India has been attributed to greening (2). The symptoms, host range, and other characteristics of these diseases are so remarkably alike as to raise the question of a common cause.

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