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International Organization of Citrus Virologists Conference Proceedings (1957-2010)

Title

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Permalink

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Journal

International Organization of Citrus Virologists Conference Proceedings
(1957-2010), 4(4)

ISSN

2313-5123

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Publication Date

1968

DOI

10.5070/C58xj5w26v

Peer reviewed

Studies on Leaf-Mottle-Yellows Disease of Citrus in the Philippines

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SINCE about 1957, great numbers of citrus trees in the Philippine Islands have died or become unproductive. Damage has been particularly important in the Province of Batangas, a leading citrus-producing region of the country, where approximately one million trees have been destroyed. Mandarins (*Citrus reticulata* Blanco) have been the most important variety, but some sweet orange [*C. sinensis* (L.) Osb.], pummelo [*C. grandis* (L.) Osb.], and calamondin (calamansi) (*C. reticulata* var. *austera* x *Fortunella* sp.) are also grown in the Islands. The principal mandarin varieties are Ladu and Szinkom. Some use is made of Batangas mandarin as a rootstock, but most of the trees are on a rootstock

known locally as calamandarin. This has not been identified botanically, but is definitely a mandarin type.

Various Philippine investigators (1, 5) made studies of the citrus tree decline in the late 1950's and early 1960's, but in several instances results were prepared only as seminar papers or Experiment Station reports and were not published (4, 6, 13). These studies demonstrated that both tristeza and seedling-yellows viruses are present in declining trees and that the efficient aphid vector *Toxoptera citricida* Kirk. is also present in the Philippines. Although these findings suggested that the tree decline could be due to tristeza, this was not proved. Furthermore, there was some circumstantial evidence that tristeza virus was not responsible for the disease which had appeared around 1957.

Indications are that in the investigations reported in 1921 by Lee (8) on mottle leaf of citrus, presumably the condition that is now known to be caused by zinc deficiency, tristeza-affected trees were studied also. Lee described and illustrated trees of mandarin and Valencia orange growing normally on mandarin rootstock and the same varieties severely diseased and stunted when grown on rootstocks of pummelo. The latter stionic combinations are now known to be intolerant of tristeza virus. Wallace *et al.* (19) presented evidence that budwood of Batangas mandarin, introduced to the United States from the Philippines prior to 1930, carried tristeza virus. Philippine Experiment Station records show that there were many introductions of citrus from India, China, and other countries in the early part of this century, and these certainly provided a means of bringing in the tristeza virus. If the assumption that the virus existed in the Philippines for many years is correct, the serious decline of citrus that occurred largely since 1957 could not be attributed solely to tristeza inasmuch as there had been no significant change in the growing of citrus from the standpoint of scion and rootstock varieties and their susceptibility to tristeza virus.

Symptomatology of Affected Orchard Trees

In the Philippines, the first symptom to appear on orchard trees is the yellowing of leaves of one or more branches. This led to the use of the name citrus yellowing by Castillo (4), who considered tristeza virus to be one of the causes of the disorder. Accompanying yellowing or developing subsequently, many leaves show leaf mottling similar to that caused by zinc deficiency. However, some leaves exhibit patterns and other effects like those resulting from deficiencies of iron, manganese, or other microelements. Nora and Baldia (14) described these and other

symptoms, but used the name tristeza for the disease because they believed that strains of seedling-yellows virus were causing the yellowing, mottling, and decline of the mandarin trees. By indexing to Mexican lime [*C. aurantifolia* (Christm.) Swing.] and sour orange (*C. aurantium* L.), they demonstrated that tristeza and/or seedling-yellows viruses were present in the affected trees included in their studies. These same authors pointed out that Nora failed to correct the yellowing and leaf mottle by application of nutritional foliar sprays.

In later stages, mottle leaf and yellowing becomes general and is accompanied by some defoliation, twig dieback, and multiple shoot growth with small, dull, chlorotic, or mottled leaves which tend to grow in an erect position. Affected trees display some of the symptoms described for greening disease in South Africa (11, 15), stubborn disease in California (2, 3, 7), and Likubin disease in Taiwan (10); such symptoms include multiple bud development, small upright leaves, and undersized, lopsided fruits. Fruits on affected trees are often more acid to the taste than normal, and color unevenly. In affected fruits, many seeds are small and dark-colored or undeveloped.

Experimental Investigations

After a visit by the junior author of this paper to the Philippines in 1963, some cooperative studies were begun in an attempt to learn the cause of the serious tree decline. It was then known that many of the declining trees were infected with tristeza virus. Thus, it was apparent that if another virus was the chief cause of tree decline, it was necessary to separate the widespread tristeza virus from the unknown virus in order for the individual effects of the two viruses to be determined. Studies were made in California of the reaction of Philippine citrus varieties to tristeza and seedling-yellows virus. Concurrently, experiments were conducted in the Philippines in efforts to learn if a causal virus other than tristeza could be identified.

Inasmuch as it seemed that it had not been firmly established that the disorder being investigated was caused by tristeza or the tristeza-seedling-yellows virus complex, the authors selected the name leaf-mottle-yellows for the disease. This name is a combination of the two most conspicuous symptoms of the disease, namely leaf mottle and yellows, and distinguishes it from leaf mottle caused by zinc deficiency as well as from the virus-induced citrus seedling yellows. In a paper in these proceedings, Salibe and Cortez (17) use the name of leaf-mottling disease for the same disorder.

Reaction of Mandarin and Other Citrus Varieties to Tristeza and Seedling-Yellows Virus Strains in California

Young seedlings of Szinkom mandarin in lots of 3-4 were inoculated with 7 virulent strains of seedling-yellows virus. Batangas mandarin seedlings were inoculated separately with 6 of the seedling-yellows strains. Calamandarin seedlings were inoculated with 3 strains of seedling-yellows virus and 1 strain of tristeza virus. All strains of seedling yellows were obtained from trees originating from importations, some as early as 1914, which came from Australia, China, Japan, Hawaii, South Africa, and the Philippines. Earlier studies by Wallace (20) presented evidence that all of these sources of seedling-yellows virus came to the United States in the original budwood. The Philippine source was derived from Batangas mandarin imported sometime prior to 1930. The date 1914, recorded by Wallace (20), is probably incorrect. The Philippine source of virus was used with the idea that it might be an unusually virulent strain of seedling yellows or that it might carry another virus which would cause leaf-mottle-yellows. However, other than slight vein clearing, none of the 3 varieties named above were affected by the inoculations.

Groups of 5 budlings of Ponkan mandarin on calamandarin stock were inoculated separately with tristeza strain 2 and a virulent strain of seedling yellows, strain 1. These budlings were not affected. A similar inoculation of budlings of Ponkan mandarin on Sunki mandarin stock produced no symptoms. Small trees of Valencia on Sunki, in lots of 4, were inoculated with 2 strains of tristeza virus and a virulent strain of seedling-yellows virus, but were unaffected. Inasmuch as both of these budded combinations are known to decline in orchard plantings in Taiwan, the results obtained suggested that the so-called Likubin disease in Taiwan is not caused by tristeza or seedling-yellows viruses as has been reported (10). Furthermore, the failure of any of the mandarin varieties to react to the virulent strains of seedling yellows used in the tests in California indicated that this virus complex is not the cause of leaf-mottle-yellows disease in the Philippines.

Studies of Declining Trees in the Philippines

TISSUE-GRAFT INOCULATIONS FROM DISEASED ORCHARD TREES.—For study in the Philippines, 5 field sources of virus were used. These are identified by number, namely, 1, 2, 3, 5, and 11.

Source 1 was obtained from a tree of Ladu mandarin on calamandarin rootstock that showed no leaf mottle or yellowing. Source 2 came from

another tree of the same combination that displayed severe leaf mottle, yellowing, and advanced twig dieback. Sources 3, 5, and 11 were obtained from different trees of Szinkom mandarin on calamandarin rootstock with severe leaf mottle, yellowing, dieback, and other abnormalities. All 5 sources indexed negatively for psorosis and exocortis, and the orchard trees from which they came showed no symptoms of xyloporosis (cachexia). All 5 sources produced a strong tristeza reaction, including vein corking on Mexican lime, and caused severe seedling yellows on Eureka lemon.

The 5 virus sources were graft-inoculated separately into 5 seedlings each of calamondin, calamandarin, Ladu and Szinkom mandarin, grapefruit (*C. paradisi* Macf.), Orlando tangelo (*C. paradisi* x *C. reticulata*), Palestine sweet lime [*C. aurantiifolia* (Christm.) Swing.], Rangpur lime (*C. reticulata* var. *austera* hyb.), Rough lemon [*C. limon* (L.) Burm. f.], sour orange (*C. aurantium* L.), sweet orange, trifoliolate orange [*Poncirus trifoliata*] (L.) Raf.], Troyer citrange (*C. sinensis* x *P. trifoliata*), and *C. macrophylla*. Several of these varieties developed one or more symptoms of tristeza such as vein clearing, stem pitting, and stunting. Sour orange and grapefruit seedlings were yellowed and stunted. This could be attributed to the presence of seedling-yellows virus because these varieties react in that manner to seedling-yellows infection. However, varieties such as Szinkom mandarin, sweet orange, calamandarin, and Rough lemon, which have not reacted to tristeza and seedling yellows in California, showed mottling and yellowing of leaves and stunting when inoculated from the 5 Philippine field sources.

Budling trees of Szinkom, Ladu, and Ponkan mandarins and sweet orange on 8 tristeza-tolerant varieties (Sunki and Cleopatra mandarin, Rangpur lime, Rough lemon, trifoliolate orange, Troyer and Carizzo citrange, and calamandarin) and 3 non-tolerant rootstock varieties (Eureka lemon, grapefruit, and sour orange) were inoculated with the 5 field sources of virus. Two budlings of each scion-rootstock combination were inoculated with each virus source and 1 budling of each kind was held as a non-inoculated control. As expected, all 5 virus sources induced typical tristeza decline on all trees of mandarins and sweet orange on the tristeza-susceptible rootstock varieties. This reaction no doubt masked other symptoms. The other budlings, with rootstocks tolerant or semi-tolerant to tristeza in North and South America, developed symptoms of leaf mottling and yellowing and became stunted. The symptoms were much like those produced by the same inocula on tristeza-tolerant seedlings.

The reactions of both seedlings and budlings inoculated by tissue-grafts from affected orchard trees in the Philippines clearly demonstrated that these trees carried either more virulent strains of the tristeza-seedling-yellows virus complex than exist in California or that they were infected also with an unidentified virus. Experiments designed to identify the virus or viruses responsible for the tree decline in the Philippines were then undertaken.

TRANSMISSION OF VIRUS FROM THE FIELD SOURCES BY THE APHID *Toxoptera citricida*.—Some transmissions were made by means of the aphid *T. citricida* to learn if virus transmitted by this vector from the 5 field sources would cause the same effects as were obtained by graft-inoculation from these sources. Martinez and Wallace (9) showed that *Aphis gossypii* Glover fed on plants carrying the tristeza-seedling-yellows complex can transmit either this complex or only the tristeza component of it. The same results occurred when *T. citricida* was used as a vector. Four infections resulting from aphid transfer of virus from the seedling-yellows field sources were studied. Three of these caused seedling yellows and 1 caused only tristeza symptoms. For identification, these were maintained as A-SY3, A-SY5, A-SY11 (aphid-transmitted seedling yellows from field sources 3, 5, and 11), and A-SYT5 (aphid-transmitted tristeza from field source 5).

These sources of aphid-transmitted virus were separately inoculated into a range of citrus selections by means of tissue-grafts. All 4 sources caused typical tristeza symptoms on indicator seedlings as well as on mandarin varieties budded on tristeza-susceptible rootstocks. Inoculations with A-SY3 and A-SY5 caused no symptoms on seedlings of numerous tristeza-tolerant varieties, but inoculations with A-SY11 and A-SYT5 caused leaf-mottle-yellows symptoms on some tristeza-tolerant varieties. These results suggested one of the following possibilities: 1) there are strains of tristeza-seedlings-yellows in the Philippines that have not been encountered in other countries where this virus complex has been studied; 2) another virus which is also transmitted by *T. citricida* causes the citrus decline in the Philippines; 3) the lime seedlings infected with A-SY11 and A-SYT5 by means of *T. citricida* became contaminated with another virus, possibly by some other vector. Results of experimental studies of these possibilities, and other investigations, insofar as they have been completed, are reported briefly in this paper.

TRANSMISSION TESTS WITH PSYLLA (*Diaphorina citri*).—Because the psylla *Diaphorina citri* Kuway is present on citrus in the Philippines in certain seasons of the year, studies were made to determine if this insect

is associated with leaf-mottle-yellows disease. In an initial trial, *D. citri* collected from nursery seedlings in the field were confined on healthy plants for 5 days and then placed on plants singly infected with the 5 sources of virus already mentioned. After 5-8 days on the virus sources, the insects were transferred to small seedlings of sweet orange and to a number of budlings of Szinkom mandarin and Valencia sweet orange. From 50 to 200 psylla were transferred to each test plant. After 5-20 days on the test plants, the insects were killed by spraying with Malathion solution. Forty sweet orange seedlings, 5 budlings of Valencia orange, and 8 budlings of Szinkom mandarin were exposed to infection by psylla. In a month following psylla exposure, 36 (90 per cent) of the seedlings and 4 (32 per cent) of the budlings developed yellowing of terminal leaves. As the growth of the test plants progressed, the leaf yellowing disappeared on many plants. However, 6 seedlings of sweet orange, 1 Valencia, and 1 Szinkom budling remained stunted, with leaves that were yellowed, slightly mottled, and reduced in size. At least 1 plant inoculated from each of the 5 field sources was permanently affected. None of the plants exposed to psylla before they had fed on the virus sources developed persistent symptoms.

Subinoculations were made by tissue-grafts from plants that developed permanent symptoms after exposure to psylla which had fed on plants that showed leaf mottle and yellows when infected by tissue-grafts from the selected orchard trees. Results proved that a virus other than tristeza was present in the diseased field trees. This virus caused leaf-mottle-yellows symptoms on seedlings of sweet orange, Mexican lime, Eureka lemon, and Batangas, Szinkom, and Sziwuikom mandarins. Budlings of tristeza-tolerant mandarin and sweet orange on rootstocks of Rangpur lime and calamandarin also developed leaf-mottle-yellows when they were inoculated with psylla-transmitted virus. Although seedlings of Mexican lime and Eureka lemon developed chlorosis and leaf mottling when infected with psylla-transmitted virus, neither of these developed characteristic symptoms of tristeza or seedling yellows. These preliminary results provide evidence that leaf-mottle-yellows in the Philippines is caused by a psylla-transmitted virus which is distinct from, and apparently unrelated to, tristeza virus and that the citrus psylla *D. citri* is a vector of this virus.*

*Salibe and Cortez reported similar findings at the 4th conference of IOCV, see p. 131. Later these same authors (16) reported that, "Observations on field spread of the disease suggest the existence of an insect vector and there is some experimental evidence that this vector is possibly *Diaphorina citri* (Kuway)."

Discussion and Conclusions

The tristeza-seedling-yellows virus complex apparently is present in most of the orchard trees in Batangas Province which show symptoms of leaf-mottle-yellows. Tissue-graft transmission from five affected orchard trees demonstrated that all carried this virus complex. These five sources of inocula caused leaf mottling, yellowing, and stunting of several kinds of citrus which have not been found to react to tristeza or seedling-yellows virus when tested in other countries.

With tristeza virus and the efficient vector *T. citricida* present in the Philippines, apparently for many years, it is to be expected that most citrus trees would be infected and that both seedling and budded trees which are susceptible to this virus would be affected. There seems to be no doubt that tristeza virus is responsible for the stem pitting that is found commonly on orchard trees of calamondin and pummelo. The decline of mandarin trees on rootstocks of Batangas mandarin and cala-mandarin, however, appears to result from other causes. In other countries, trees of sweet orange and mandarin on mandarin rootstocks are highly tolerant of tristeza, and experiments in California demonstrated that cala-mandarin is also a tolerant rootstock.

The demonstration that declining trees in the Philippines carry a virus that can be transmitted by the citrus psylla *D. citri*, which does not cause typical tristeza symptoms on Mexican lime or seedling yellows on Eureka lemon, certainly indicates that this virus (LMYV) is the cause of leaf-mottle-yellows disease. However, in these studies it was found that two sources of virus obtained by aphid transmission from leaf-mottle-yellows trees caused leaf mottle and yellows when graft-transmitted to varieties which normally are not affected by tristeza or seedling-yellows virus. This indicated that until further studies are made, tristeza and seedling-yellows viruses cannot be completely eliminated as causes for at least some of the decline in the Philippines. Experiments, now in progress, in which aphids and psylla are being tested, respectively, as vectors of aphid-transmitted and psylla-transmitted viruses should identify specifically the causal virus of leaf-mottle-yellows disease and demonstrate whether or not tristeza and seedling-yellows viruses and/or their vector, *T. citricida*, have any relationship to the disease.†

†Since presentation of this paper at the 1966 IOCV conference it has been determined that one of the aphid-transmitted virus sources (SYT5) which caused symptoms of both tristeza and leaf-mottle-yellows consisted of a mixture of tristeza virus and a second virus. When psylla were fed on SYT5-infected plants they transmitted virus which caused leaf-mottle-yellows symptoms only. It has not been determined

Leaf-mottle-yellows in the Philippines appears quite similar to the greening disease in South Africa which is transmitted by a citrus psylla, *Trioza (Spanioza) erythrae* Delg. (11, 12, 15, 18). The relation of these two diseases to each other as well as their relation to such diseases as stubborn and Likubin may be determined only when proper comparative studies are made.

Leaf-mottle-yellows virus disease has caused great losses of citrus trees in the Philippines. The virus attacks both seedling and budded trees. From preliminary investigations it seems that none of the principal citrus varieties grown for fruit production show a high degree of resistance. Trifoliate orange and Troyer citrange appear to be highly tolerant of LMYV when inoculated as seedlings. However, as rootstocks, they conferred no tolerance to the scion growth of Philippine mandarin varieties. The writers do not consider leaf-mottle-yellows to be a scion-rootstock disease and are of the opinion that control will necessitate the finding of tolerant scion varieties. Quarantine measures may aid in restricting the spread of the disease from affected to non-affected citrus regions.

Leaf-mottle-yellows disease is a potential threat wherever citrus is grown. The urgency of further study of this serious disorder, with emphasis on control or prevention, is obvious.

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how the LMYV got into the plant that was inoculated by aphids, but this occurrence and other tests suggest that *T. citricidus*, under some conditions, may transmit LMYV. Other experimental investigations completed by the authors after preparation of this paper, involving study of virus-vector relationships, host range, varietal reactions, and cross-protection phenomena, permit the conclusion that a psylla-transmitted virus is the cause of leaf-mottle-yellows disease. Possible transmission of LMYV by the tristeza vector, *T. citricida*, is being investigated.

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