CITRUS GREENING DISEASE IN THE PHILIPPINES

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INTRODUCTION

This paper refers to a disorder of citrus trees which has been known in the Philippines as "yellows," "leaf mottling disease" and "leaf-mottle-yellows disease." From information already published and other data presented in this paper the writers are of the opinion that the name "greening" should be used for the disease in the Philippines. Except for transmission by two different species of citrus psylla, neither of which is present in both countries, the citrus dieback disease in India is now considered to be the same as greening disease in South Africa. Consequently, the name "greening" is now being used for the disease in India. Inasmuch as the syndrome of the affected trees in India and the Philippines is the same and because in both countries the causal virus is transmitted by the same species of psylla it seems desirable at this time to describe the Philippine disorder as greening disease.

HISTORY, IMPORTANCE AND DISTRIBUTION IN THE PHILIPPINES

Although citrus has been grown to some extent in the Philippines for a long time, the major expansion of citrus acreage took place after World War II with the plantings consisting for the most part of mandarins, pummelo, calamondin and some sweet oranges. In these later plantings, budded trees were grown largely on a rootstock known there as calamandarin. The exact origin of calamandarin is not known nor has it been identified botanically. It is a mandarin type which resembles Cleopatra mandarin.

In the past decade the disease which we have referred to has affected and rendered unproductive a great number of citrus trees in the Philippines, particularly in the provinces of Batangas, Laguna and Quezon on the island of Luzon. The devastating effects of the disease were not recognized fully until 1960 when surveys showed that thousands of citrus trees were in rather advanced stages of decline. In subsequent years continuous severe tree losses have been experienced in the Batangas area. Whether the disease is also present in other important citrus-growing areas of the Philippines which include central and northern Luzon, Bicol, Visayas and Mindanao, has not been determined with certainty. However, because most of the planting material in those regions originated from Batangas, it is likely that the virus is present wherever citrus is being grown.

The total losses resulting from greening disease in the Philippines have not been determined because of lack of complete surveys and systematic study in some of the citrus-growing areas. However, the most serious damage has occurred in the Province of Batangas. Studies by the Division of Agricultural Economics gave an estimate of over seven million citrus trees in the Philippines in 1962. It is now generally accepted that more than one million trees have been lost specifically from greening disease. Some of the heavily devastated citrus orchards in Batangas Province are now abandoned and other citrus acreage has been replaced by rice, corn, sugarcane, coconut and vegetables.

The origin of the greening disease in the Philippines is not known. It is certain that there were many introductions of budwood and small budded trees from mainland China, India, Japan and Taiwan prior to 1957. It seems likely that the greening virus was brought into the Philippines in some of these citrus introductions.

Following World War II there was a great increase in citrus acreage in the Philippines, especially in Batangas Province. During that time there was an opportunity for rather general distribution of infected trees and further spread of the greening virus by its vector no doubt followed.

SYMPTOMATOLOGY

The symptoms of greening disease in affected orchard trees in the Philippines are conspicuous yellowing and mottling of the leaves, defoliation and die-back of twigs and branches, and production of multiple buds and abnormal fruits. The leaves of secondary growth arising from multiple buds are completely yellowed and mottled, small, upright, leathery, narrow in relation to length and in some cases, the leaf tips tend to curl downward. Affected fruits are abnormally small, under-developed, lopsided, strongly acid in taste, and somewhat hardened. Some fruits exhibit yellowing of the stem-end and the peduncle assumes a brownish discoloration. Premature fall of many fruits at different stages of development is common. In some cases, particularly on Szinkom mandarin, some of the fruits produced on severely yellowed and mottled branches become dry or mummified and remain firmly attached to the affected tree. Some of the seeds produced in abnormal fruits appear normal in size and germinate into vigorous seedlings but others are small, under-developed, dark colored and aborted.

Some affected trees are completely yellowed and mottled while others have only one or more branches showing these symptoms. After symptoms appear on one part of the tree, they later develop quite generally and affected parts then begin to defoliate with subsequent die-back and general decline.

Limited observations revealed that some young trees on calamandarin rootstock in the Batangas area collapsed before their bearing age, resembling the quick-decline type of tristeza. Frazer, D. Singh, Capoor and Nariani (1966) observed some sudden wilting of orchard trees in India followed by death of the trees.

EXPERIMENTAL INVESTIGATIONS

Since 1957 studies on various aspects of the disease have been made to determine the cause of the tree decline. Because affected trees displayed symptoms of nutritional disorders Nora (1961) applied nutritional elements but only slight and temporary improvement of diseased trees resulted. Also, other workers (unpublished) reported that fungi and/or nematodes were not the cause.

The tristeza virus is known to have existed in the Philippines many years before 1957 (Wallace, Oberholzer and Hofmeyer, 1956). Studies by Celino (1961), Nora (1961), and Nora and Baldia (1962) demonstrated that the tristeza-seedling yellows virus complex and the aphid vector *Toxoptera citricida,* Kirk. are present in the Philippines. Since that time this has been substantiated by numerous investigators and it is now known that these viruses are generally present in the declining trees (Martinez, Nora and Sebastian, 1965).

When the authors began study of the citrus decline in the Philippines in 1963 it was not known if trees on calamandarin rootstock are affected by tristeza virus. One of the first experiments conducted was to experimentally determine this point. Trees of mandarin and sweet orange on calamandarin rootstock were grown and inocualted in California with virulent strains of tristeza and seedling-yellows viruses. These tests, conducted under controlled conditions and in the absence of other viruses, demonstrated that such trees on calamandarin rootstocks were not affected by the tristeza-seedling yellows viruses. It was further determined that these viruses did not affect certain Philippine mandarin varieties when they were infected as seedlings. These studies, reported by Martinez and Wallace (1967), made it evident that unless different and more virulent strains of tristeza-seedling yellows viruses were present another virus was responsible for the decline of trees in the Philippines.

This paper reports largely on the investigations of the authors. Except for one paper by Salibe and Cortez (1966) and the paper of the authors cited above, details of other studies have not been published.

Tissue Graft Inoculations–The first transmission tests made by the authors were concerned with graft inoculations from affected trees. Five orchard trees in varying stages of decline were selected as sources of inocula. Graft inoculations to Mexican lime and Eureka lemon showed that all five trees carried the viruses of the tristeza-seedling yellows complex. However, inoculations to both seedling varieties and budlings of varietal combinations which are tolerant of tristeza resulted in mottling, yellowing and stunting. Salibe and Cortez (1966) reported similar results of graft transmission experiments.

Propagations from affected orchard mandarin trees were made on tristeza-tolerant rootstock varieties such as sweet orange, mandarin, trifoliate orange, Rough lemon, *et cetera*. In some instances the scion growth was mottled and yellowed but sometimes it developed normally. Several of the normally-growing scions were tested and found to be infected with seedling-yellows virus. These results suggested that another virus was the cause of the leaf-mottle symptoms and that it was not evenly distributed in orchard trees or at least that it was not present in all buds.

Because all field sources of inocula being studied, tested positively for seedling-yellows virus, efforts were directed early in the investigations to obtain and study this virus complex isolated from any other virus which might be present. Also it was desirable to obtain, if it existed, the other suspected virus free of tristeza-seedling yellows. Until these two things could be done it was not possible to establish the exact cause of the tree decline. Because tristeza virus commonly fails to infect trifoliate orange, this offered a possible means of obtaining the unknown virus free of tristeza contamination. Aphid transmission from the orchard virus sources provided a possible means of obtaining sources of tristeza virus not mixed with the other virus if it was present along with tristeza virus in the declining trees. Experiments were then initiated to accomplish both objectives.

Transmission Through Trifoliate Orange–In the first tests of trifoliate orange seedlings which had been inoculated with the field sources of virus there was no recovery of either tristeza virus or an accompanying virus which caused leaf mottle on mandarins and other varieties. This suggested that the virus that caused leaf-mottle symptoms was possibly a strain of tristeza or a related virus. However, in further trials it was found that some of the inoculated trifoliate orange seedlings yielded a virus which caused no tristeza or seedling-yellows symptoms respectively on lime and Eureka lemon but produced the typical leaf-mottle disease on mandarins and other varieties.

Transmission Tests with Toxoptera citricida–Aphids which fed on young plants previously infected with field sources of virus were transferred to seedling limes. Several plants developed symptoms of tristeza. When these were tested further on Eureka lemon it was found that some of these caused seedling yellows and others caused only tristeza, as had been reported earlier by Martinez and Wallace (1964) in studies of seedling yellows in California. When the aphid-transmitted virus sources were inoculated into varieties which normally do not react to tristeza or seedling yellows, some had no effect but two of them caused typical leaf mottle. Subsequent studies of one of these proved that it was a mixture of tristeza and greening viruses. The other source also may have been a mixture of these two viruses but it was not maintained for further study.

As is discussed in the following section it is now known that a citrus psylla, *Diaphorina citri* Kuway is a vector of the virus which is responsible for the decline of Philippine citrus

under consideration in this paper, a disease we conclude to be greening. With the discovery of this vector it has been possible to obtain sources of greening virus which are not mixed with tristeza virus for use in further studies of possible transmission of greening virus by the aphid, *Toxoptera citricida.* It can be reported at this time that some test plants, after exposure to aphids which had been given an acquisition feeding on plants infected only with psylla-transmitted virus, developed mild leaf mottle and slight stunting. None of these developed severe greening symptoms but graft transmissions from them to other plants resulted in similar symptoms. This is being given further study in an attempt to determine if possibly a mild strain of greening virus is sometimes transmitted by *Toxoptera citricida.*

Transmission by the Citrus Psylla, Diaphorina citri–It is now established that the citrus psylla *Diaphorina citri* transmits the virus that causes the decline of tristeza-tolerant trees in the Philippines. In our studies, infections have resulted from psylla that fed on tristeza-tolerant plants which had developed leaf mottle, yellowing and stunting after being graft-inoculated from declining orchard trees. Although tristeza and seedling-yellows viruses were present in the plants on which the psylla fed, neither of these were transmitted by the psylla. Also, after the field sources of virus were passed through trifoliate orange the remaining virus was transmitted by psylla and caused leaf mottling, yellowing and stunting on citrus varieties which do not react to tristeza virus. It is concluded that this is the greening virus.

Symptoms of Greening on Experimentally-Infected Plants–Under greenhouse conditions citrus seedlings and budlings of different kinds experimentally infected with greening virus exhibit symptoms of varying degrees of severity. In general, the earliest visible symptoms on these plants are mild yellowing and slight reduction in size of the terminal leaves. Yellowing of the terminal leaves becomes more consipcuous as the plants advance in age. Then the leaf tissue immediately adjacent to the midvein and some lateral veins remains yellowish green to dark green in appearance. The subsequently developed leaves show symptoms that are essentially the same as the foliar symptoms of affected orchard trees already described. Yellowing of the leaves is rather complete or there are some green areas along the midvein and lateral veins which are suggestive of zinc, manganese and iron deficiencies. In some varieties there is yellowing of the midvein and lateral veins. Severely infected plants are stunted in growth. They have a rather poor root system. They remain dormant for sometime and then produce secondary growth that is yellowed and mottled, bushy, and upright. Partial to complete defoliation and die-back of some twigs is common. Some plants died within 6 - 8 months after being infected.

Varietal Reactions–Tests on the reaction of different citrus varieties and hybrids and citrus relatives to greening virus were conducted inside the greenhouse. Test plants were seedlings as well as budlings on various rootstock varieties, with each inoculated plant receiving three buds from infected plants. Varieties and hybrids that showed yellowing and mottling and great reduction in size of the leaves and severe stunting are Szinkom, Sziwuikom, Sunwuikom, Batangas, Ladu, Calamandarin, Ransas, Cleopatra, Ponkan, Oneco, King, Avana, Malvasio, Murcott Honey, Madam Vinous, Pera, Hamlin, Koethen, Shamouti, Caipira, Campbell Valencia, Washington navel, Orlando tangelo, Szinkom x Batangas, Szinkom x Ladu, Szinkom x King, Shekwasa x Calamondin and an unidentified variety of sweet orange. Those that showed mild to moderate yellowing and diffused mottling and moderate stunting are grapefruit, sour orange, Sunki mandarin, Rough lemon, Rangpur lime, Borneo red lime, Eureka lemon, Key lime, Palestine sweet lime, Shekwasa, native and Siamese pummeloes, Etrog citron 60 - 13 and 861, and two unknown varieties of citron. Those that showed slight yellowing and faint mottling and slight stunting are Calamondin, *Citrus macrophylla,* and *Severinia buxifolia.* Those that showed no leaf symptoms are trifoliate orange, Troyer and Carrizo citrange, *Aeglopsis chevalieri* and *Atalantia trifolia.* Subinoculations from these symptomless plants demonstrated that all five can serve as hosts of greening virus.

When trifoliate orange and Troyer and Carrizo citranges were used as rootstocks for Ladu, Ponkan and Szinkom mandarin and Madam Vinous, Campbell Valencia, and Washington navel oranges and these budlings were inoculated with psylla-transmitted virus all of these fruit varieties showed symptoms that were as severe as when these varieties were inoculated as seedlings (Martinez, 1967). Thus it has been demonstrated that the virus affects the scion varieties directly and that the tolerant rootstocks conferred no resistance to the scion.

Mechanical Transmission Tests–Schwarz (1966) reported successful transmission of greening virus in South Africa from citrus to cucumber and from cucumber back to citrus. In the Philippines repeated efforts to transmit greening virus to citrus and to cucumber, cowpea, beans, squash, spinach, sesame, periwinkle, tobacco (*N. glutinosa*), Chenopodium and Gomphrena gave negative results. Further efforts are being made to transmit the virus mechanically.

Cross-Protection Tests–Mandarin and sweet-orange plants infected separately with the viruses of tristeza, seedling yellows, exocortis, psorosis, and xyloporosis were challenge-inoculated with greening virus. None of these viruses afforded protection against greening virus. In fact it appeared that some of the doubly-inoculated plants reacted more severely than those having only the greening virus. This led to further investigations on possible synergistic reactions.

Synergistic Reactions–Budlings of Ladu, Ponkan and Szinkom mandarin on Calamandarin rootstocks, five of each combination, were treated as follows:

a. Inoculated with greening virus
b. Inoculated with tristeza virus
c. Inoculated with seedling-yellows virus
d. Inoculated with greening and tristeza viruses simultaneously
e. Inoculated with greening and seedling-yellows viruses simultaneously
f. No inoculation

There was a total of 15 plants in each group. The greening virus used had been established by transmission by psylla and the tristeza and seedling-yellows virus sources had

been obtained by aphid transfer. The plants inoculated only with tristeza or seedling-yellows viruses were unaffected. Those infected only with greening virus developed severe leaf mottle, yellowing and stunting. The plants inoculated with greening plus tristeza virus and greening plus seedling-yellows virus reacted initially like those inoculated only with greening virus but 21 out of 30 trees in the doubly-infected groups collapsed before any of the 15 trees with greening virus alone reached that stage of decline.

In the Philippines, as reported by Fraser *et al.* (1966) in India an occasional young tree in field plantings has been observed to collapse and die rather suddenly. Possibly such trees were doubly infected with greening and tristeza viruses. It is known that the viruses of tristeza and seedling yellows have some injurious effects on the so-called resistant or tolerant fruit varieties. Thus, when these viruses are present in combination with greening virus the total effect may bring on a faster or even more severe reaction.

DISCUSSION AND CONCLUSIONS

It is concluded that the virus-induced disease which has been responsible for the great loss of citrus trees in the Philippines since the 1950's is identical to the so-called citrus dieback in India and that very probably it is the same as greening disease first described from South Africa. Presently the only firmly established difference between the disease in the Philippines and that in South Africa seems to be that the two are transmitted by different species of citrus psylla. The known Philippine vector *Diaphorina citri* has not been found in South Africa and the South African vector *Trioza erytreae* has not been reported in the Philippines.

In tests so far conducted the Philippine virus has not been mechanically transmitted to cucumber as has been reported for the greening virus in South Africa. However, with citrus viruses, mechanical transmission often requires special techniques and proper environmental conditions.

Certainly it appears that the psylla-transmitted virus which causes the citrus decline in India is the same as the virus that is responsible for the great loss of trees in the Philippines. The authors make this conclusion and for the sake of uniformity, suggest that the disease in the Philippines should now be referred to as "greening" disease.

It seems likely that the "vein-phloem degeneration disease" described by Tirtawidjaja, Hadiwidjaja and Lasheen (1965) in Java and the "li-ku-bin" disease in Taiwan (Matsumoto, Wang and Su, 1961; Wallace, 1963) also will prove to be the same as greening but further investigation of this relationship is needed.

It now appears that in regions where both the greening virus and its vector are present, this disease has the potentiality of destroying all existing citrus orchards. The apparent lack of resistance in the commercially-grown fruit varieties suggests that successful control or prevention will require other measures. The search for tolerant varieties should be continued but at the same time other means of control must be investigated. The authors suggest that in addition to other studies some research efforts should be directed towards the discovery of mild, protective strains of greening virus and the control of insect vectors.

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