Virus Diseases of the Citrus and Researches Conducted on Them in Japan

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In Japan the total area of citrus plantation extends over about 140,000 hectares, among which about 120,000 hectares are covered with the Satsuma mandarin. The citrus fruit produced in this country is consumed, for the most part, in the raw, while a part of which is made into canned fruit or juice. It is numbered among the important exports in Japan.

Citrus trees, which are cultivated in the comparatively warmer and rainy districts in Japan, are often damaged by various fungal diseases so studies on the fungal diseases have been carried out for a long time. The virus disease, however, was taken up for study rather recently around 1948.

There are several disorders which show characteristic symptoms and are described as virus diseases. During the studies on these diseases, it has also been made clear that healthy appearing trees often carry viruses in latent condition. In the following, symptoms of the main virus diseases of citrus trees in this country will be described with the present state of studies on them.

Satsuma Dwarf and its Relative Virus Diseases

In this country, four virus diseases including Satsuma dwarf are known to be of this number. Their pathogens had been thought the psorosis virus from the various symptoms of young leaves observed in inoculated seedlings of several species of citrus, transmission to many herbaceous plants by sap inoculation and, moreover, electron microscopic figures of the pathogenic viruses.

a. *Satsuma dwarf* This is the first virus disease of the citrus tree studied in Japan in 1948. It appears to distribute widely in the Satsuma mandarin-cultivating areas, though we have no detailed data on the distribution of this disease through the country. [8], [13] Th

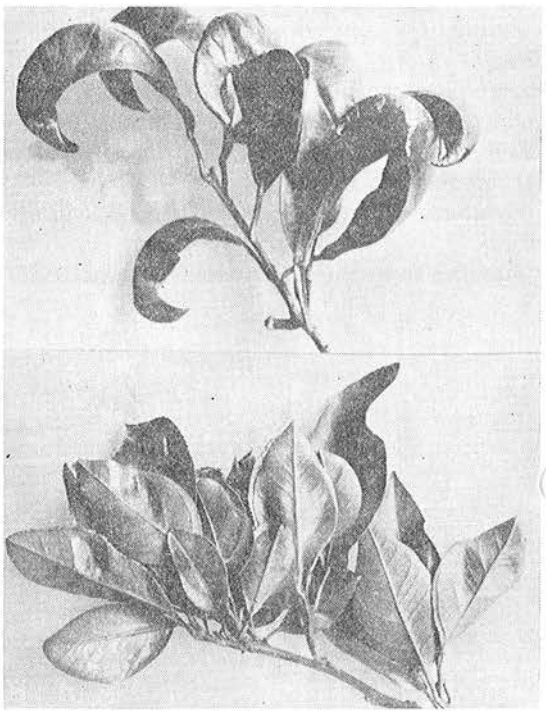

Fig. 1. Boat-shape (upper) and spoon-shape (lower) mature leaf symptoms of Satsuma mandarin leaves affected with Satsuma dwarf (crinkly leaf virus ?)

typical symptom is boat or spoon-like malformed leaves. The disease is chronic causing poor growth of trees, gradual lowering both in quality and quantity of fruit production. Appearance

of the malformation is limited to spring flush leaves in natural conditions. It has been experimentally made clear that the appearance of symptom is affected by the temperature at the time of flushing, being masked at exposure to temperatures above 28°C. for about 12 hours per every day [7].

Sesame (*Sesamum indicum*) or leguminous plants are known to be suitable test plants for this virus. That is, when sap inoculation is made on seedlings of those plants by the Carborundum method, sesame seedlings show such symptoms as local lesions of the treated leaves, and vein clearing, vein necrosis or curling and malformation of the non-treated upper leaves several days after inoculation, while leguminous seedlings manifest mottlings and vein necrosis limited to the upper leaves and stem necrosis. [2),3),9),11)]

Such a virus as manifests the characteristic symptom mentioned above in sesame and leguminous plants was described as Satsuma dwarf virus, and is thought to be the pathogenic virus of this disease. [9)] Investigations in recent years, however, show that this virus causes crinkle in the leaves of seedlings of various citrus species and is transmitted to citrus seedlings by sap inoculation, so the Satsuma dwarf virus is now regarded as the citrus crinkly leaf virus described in the other countries or its near relative. According to a report on the electron microscopic observations the virus particle is icosahedral in shape and about 26 mμ in diameter. Physical properties of the virus such as thermal inactivation point, dilutioned point and aging *in vitro* have also been studied. [4)]

The virus is known to be spread by grafting and through the sap of diseased plants. Experiments on the possibilities of spread through roots, with pollen grains and seeds are now in progress. The green broad-winged planthopper (*Geisha distinctissima*) was once reported to be a vector of this virus, but it seems to be a mistake. Whether there are insect vectors of this virus or not is unknown at present. The main point of the countermeasures to this disease is to prevent the spread with nursery-trees, so the governmental inspection of the mother trees from which scions are to be taken is in force.

b. *Virus diseases relative to Satsuma dwarf*

The undermentioned diseases are dealt with as relatives to the Satsuma dwarf, because they have such common features as are positive in the sesame reaction and induce boat-shape and crinkled leaves in virus-free seedlings of the Satsuma mandarin after graft inoculation.

i. Citrus mosaic: This is an endemic disease which occurs only in a limited area of Wakayama Prefecture for the present. The symptom is that ring-shaped spots appear at a portion of the surface or on the whole surface of fruit at about the stage of coloring, and coloring is delayed at the spotted part. Leaves show

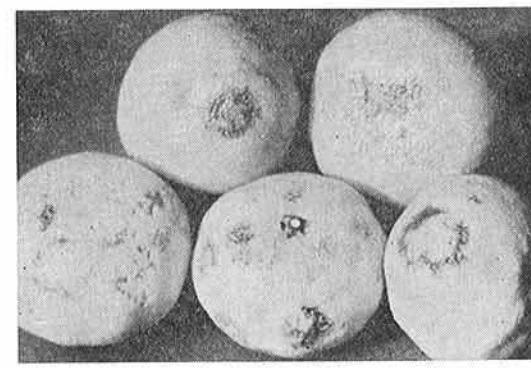

Fig. 2. Ring type mature fruit symptoms of Satsuma mandarin affected with mosaic (infectious variegation virus?)

malformation like the symptom of Satsuma dwarf. [1),8)]

ii. Natsudaidai dwarf-A: This disease is not widely distributed, occurring only in a limited area of Yamaguchi Prefecture. The symptom is mottling and curling of spring flush leaves. The fruit becomes smaller in size and less in production. [8)]

iii. Navel infectious mottling: This occurs in a limited area of Wakayama Prefecture. The typical symptom is mottling of leaves and browning in the chlorotic area of their under surface. Fruit is smaller in size and in amount of harvest.

The above-mentioned three diseases cause variegation, crinkling and remarkable narrowing of leaves or distinct yellowing of new flush on seedlings of various citrus species inoculated by grafting. They can also be communicated by

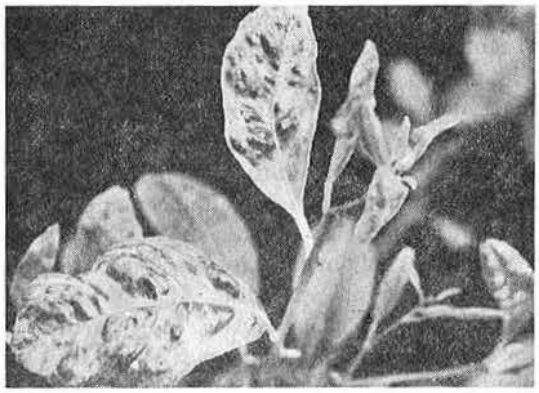

Fig. 3. Crinkle and variegation of rough lemon leaves inoculated with Natsudaidai dwarf-A (infectious variegation virus ?)

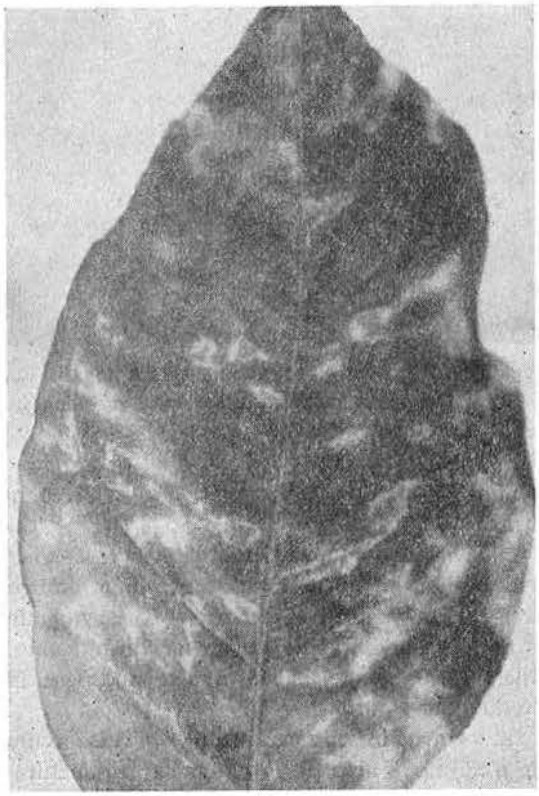

Fig. 4. Ring type mature leaf symptoms of Washington navel orange affected with infectious mottling (infectious variegation virus ?)

sap inoculation. Judging from these facts, the pathogenic virus of these disease is considered to be the citrus variegation virus or its near relatives. [15]

Hassaku Dwarf and its Relative Virus Diseases

Such diseases show no specific symptom but the tristeza reaction in the Mexican lime is included in this group. The pathogen is considered to belong to the tristeza virus from the reaction observed on the Mexican lime seedling and electron microscopic figures of the virus.

a. *Hassaku dwarf* Next to the Satsuma mandarin and Natsudaidai, the Hassaku is the most important citrus in this country, being cultivated mainly in Hiroshima Prefecture. Hassaku dwarf is the most severe one among the virus diseases of the citrus in Japan. Symptoms are pitting appearing on the branch and

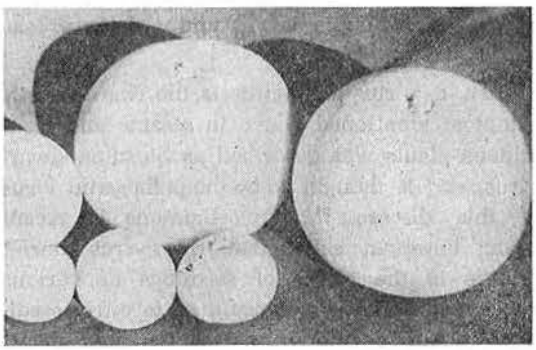

Fig. 5. Healthy (right) and affected (left) mature fruits of Hassaku dwarf (tristeza virus)

trank, smaller leaves in size, remarkable poor growth, and smaller fruit losing market value.

The Mexican lime and Marumera (*Citrus obovoidea*) are suitable for testing this disease. The seedlings of those plants inoculated by grafting show very severe symptoms known as the tristeza reaction, that is, vein clearing, stem pitting and vein corking accompanied with remarkably stunting. [10], [11]

The pathogen is considered, as mentioned above, to be the tristeza virus. There are two strains of the virus, severe and mild, and the severe damage on the Hassaku seems to be done by the former. [6] Electron microscopic observations revealed that the virus is string-like in shape as the tristeza found in the other countries.

It is known that this virus is transmitted by grafting and also by an insect vector, tropical

citrus aphid (*Toxoptera citricidus*). [5]

At present there are no Hassaku trees which are free from the virus, therefore production of nursery-trees is carried out by selecting mother trees which seem to carry only the mild strain of virus and show very slight or no symptom of the disease. As the tristeza is the pathogenic virus, experiments have also been carried out for selecting root-stocks tolerant to it, obtaining the results that the rough lemon and Cleopatra mandarin appear to be promising for this purpose. Further experiments, however, are required for the practical use of these tolerant root-stocks.

b. *Natsudaidai dwarf-B* This disease occurs in Ehime and Oita Prefectures, etc. The symptom is similar to that of the above-mentioned Natsudaidai dwarf-A except the appearance of pitting on the branch and trank, and no mottling of young leaves. The pathogenic virus, however, is considered to be the tristeza as in Hassaku dwarf, because its reactions to various test plants are only vein clearing and corking, and completely different from that of Natsudaidai dwarf-A.

Exocortis

As most of the citrus trees are of trifoliate root-stock in Japan, precaution must be taken against the spread of the exocortis virus. In Japan investigations on this virus started around 1963. And its existence in this country was confirmed by quick tests with the Etrog citron and inoculation experiments in the trifoliate and Rangpur lime seedlings, [16] or by color tests with phloroglucinol-HCl reagent. [12] It, however, appears to exist only in the citrus trees brought from foreign countries and kept in Horticultural Research Station and others, having never been found in the Satsuma mandarin and other citrus trees under commercial cultivation until now.

Fig. 6. Exocortis on trifaliate root stock of Marsh grapefruit.

The symptom is as same as described in the other countries, that is, cracking or scaling of the bark of trifoliate root-stocks causing remarkably inferior growth and decrease in the amount of harvest.

Latent Viruses of the Citrus Tree

a. *Tristeza virus* Extensive examinations carried out using the Mexican lime as the test plant revealed that this virus is found in almost all citrus trees in Japan but young seedlings,[10] though the virus appears to have practically no important effect upon the productivity of trees. Citrus trees are usually propargated on trifoliate or yuzu (*Citrus junos*) rootstocks in Japan, therefore the resistance of these trees to the virus seems to come from their scion-rootstock combination. This was confirmed by inoculation experiments of tristeza virus to virus-free Satsuma mandarin nursery-trees, which were propagated on trifoliate, yuzu or sour orange rootstocks. The Satsuma mandarin on sour orange shows rapid decline in growth and yellowing of leaves after inoculation, while the tree on trifoliate or yuzu is nearly normal, showing little effect of inoculation[14].

b. *Vein enation virus* When examinations are made using the rough lemon or sour orange as test plants, enation is often observed on vein or veinlet of leaves of the test plants inoculated with Satsuma mandarin and Hassaku, etc., showing that this virus is rather widely distributed in Japan.[8] Enation is also found commonly on leaves of Kawabata-mikan (*Citrus aurea*) and rarely on Natsudaidai and Kabusu (*C. aurantium*) under natural conditions. The actual damage by this virus, however, is not clear at present.

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