

A New Citrus Virus Disease: Citrus Yellow Mottle

Kinji Ushiyama, Tomio Usugi, and Hiroyuki Hibino

ABSTRACT. Distinct vein clearing with yellowish halos were found on leaves of satsuma mandarin trees in Japan. The causal agent of the symptoms was transmitted to young satsuma mandarin trees and other citrus seedlings by graft inoculation. Rod-shaped particles mostly 690-740 nm X 12-14 nm were detected in partially purified preparations and in leaf-dips from leaves showing symptoms. Masses of similar rod-shaped particles were also observed in ultra-thin sections of affected leaves. Mechanical transmission to herbaceous plants and citrus seedlings using crude sap were unsuccessful. However, back inoculation by knife stem-cut method of the 100,000xg sediment fraction prepared from the affected citrus seedlings to Fukuhara sweet orange seedlings was successful. These results indicate that the rod-shaped particle is the causal virus of this new disease of citrus. The name citrus yellow mottle and citrus yellow mottle virus (CYMV) are proposed for the disease and its virus agent, respectively.

Distinct vein clearing with yellowish halos were found on the leaves of satsuma mandarin trees in Kanagawa Prefecture of Japan in 1968. Subsequent surveys to the present revealed only three affected trees in two citrus orchards. One tree in Odawara was a satsuma mandarin approximately 40 years old top-worked with Wase-satsuma and showed severe symptoms on the leaves of some branches. The other two trees in Yugawara were approximately 60 year-old satsuma mandarin trees and exhibited scattered symptoms on some branches. Symptoms of the disease somewhat resembled those of citrus ringspot originally described by Wallace and Drake (14) and reported from many citrus-growing areas of the world (2, 4, 5, 8, 13). The causal agent of citrus ringspot has been transmitted from citrus to herbaceous plants by mechanical means (4, 5, 6, 10, 11, 12) and from citrus to herbaceous plants and herbaceous plants to citrus by dodder (3), however, its morphology has not yet been described.

In this paper, symptoms of this newly discovered disease, its transmission, and the morphology of the causal agent are reported.

MATERIALS AND METHODS

Inoculum source tree. The dis-

eased tree in Odawara was usually used as the virus source.

Graft inoculation. Nursery trees of satsuma mandarin and other varieties of citrus seedlings were used for graft inoculation tests. These were planted on steam-sterilized soil in earthen pots and kept in a glasshouse at temperatures below 28 C from April to October.

Mechanical inoculation. Young leaves from the original source tree were mainly used as the inoculum source in May, and those from graft-inoculated citrus seedlings having symptoms were sometimes used during March and August. These leaves were ground in a porcelain mortar in 5 to 10 parts of cold 0.05 M potassium phosphate buffer, pH 7.00, alone or containing 0.1% thioglycolic acid, or 0.05 M tris-HCl (tris (hydroxymethyl) aminomethane) buffer solution, pH 8.00, containing 0.5% 2-mercaptoethanol. The extracts were inoculated to young, tender leaves of herbaceous and citrus seedlings with an absorbent cotton swab by the carborundum leaf abrasion method. After inoculation, plants were kept in an air-cooled and partly shaded glasshouse at temperatures below 25 C.

Partial purification. Fukuhara sweet orange seedlings which were graft-inoculated with buds of the

original diseased tree and showed severe leaf symptoms were used as a tissue source. Two or three g of young leaf tissue were ground in a porcelain mortar with 10 volumes of 0.05 M potassium phosphate buffer, pH 7.00. Sap was expressed through gauze, and thoroughly shaken with 1/5 volume of carbon tetrachloride at 5 C for 15 minutes with a magnetic stirrer. After centrifuging at 2,700xg for 15 minutes, the supernatant was centrifuged again at 100,000xg for 60 minutes. The pellet was re-suspended in about 0.5 ml of potassium phosphate buffer and used for electron microscopy and for stem-cut inoculation.

Electron microscopy. To make leaf-dip preparations, cut surfaces of symptomatic young leaf tissues were dipped several times in drops of 2% phosphotungstic acid in water and adjusted to pH 7.0. Some preparations were made in distilled water and shadowed with chromium. Preparations obtained by partial purification and leaf-dipping were observed for virus particles using a Hitachi H-500 electron microscope. Particles were measured on negatives. To make preparations for observation of fine structure in ultrathin sections, small leaf pieces showing symptoms were fixed with glutaraldehyde, postfixed with osmium tetroxide, and embedded in Epon resin. The thin sections were cut by a diamond knife. The sections were stained with uranyl acetate and lead citrate, and examined under an electron microscope (Hitachi H-500).

Stem-cut inoculation. Partially purified preparations were used to inoculate Fukuhara sweet orange seedlings by cutting five areas of the stems ten times with a knife blade moistened with inoculum. Six seedlings in October, 1979 (trial 1) and five seedlings in May, 1980 (trial 2) were used for inocu-

lation tests. They were kept in a glasshouse at a temperature less than 28 C until May, 1981, when leaf symptoms appeared. Inoculated Fukuhara sweet orange seedlings were indexed by budding to Mexican lime seedlings for citrus tristeza virus (CTV) infection. Furthermore, CTV infection in leaves of Fukuhara sweet orange and Mexican lime was tested by enzyme-linked immunosorbent assay (ELISA) (1).

RESULTS AND DISCUSSION

Disease symptoms. Distinct and somewhat large vein clearing (fig. 1B) on leaves with surrounding yellowish halos were characteristic of the disease. These leaf symptoms usually appeared on some branches of the trees. Vein clearing appeared in young leaves and persisted as they matured. Some branches were severely affected and showed symptoms similar to zinc deficiency (fig. 1A), however, others remained quite healthy. No symptoms appeared on shoots and fruits, and the trees were not stunted.

Graft inoculation. When satsuma mandarin seedlings and nursery trees were graft inoculated, faint vein clearing and chlorotic spots appeared on young leaves. Later, they gradually became large and conspicuous and turned yellowish. When the leaves matured, they showed the same symptoms observed in the naturally diseased satsuma mandarin trees. Leaves of Mexican lime seedlings inoculated with buds of the affected field trees usually showed severe symptoms of CTV such as vein clearing and vein corking. To eliminate the influence of CTV on citrus indicator seedlings (7), the virus was transmitted to trifoliate orange and buds from the infected trifoliate orange were used as an inoculum source. Vein clearing, yellowish ring spot or blotches and occasional-

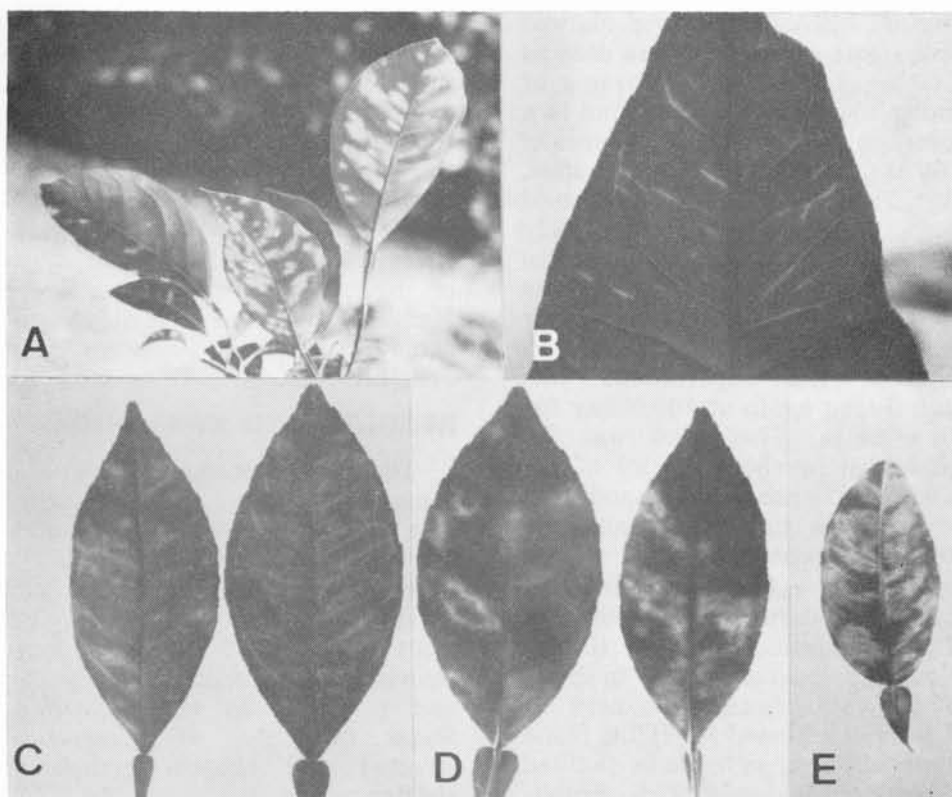


Fig. 1. Symptoms of citrus yellow mottle. A) Distinct vein clearing with yellow halos on leaves of diseased trees of satsuma mandarin. B) Vein clearing on mature leaf of satsuma mandarin. C, D) Vein clearing or vein yellow type and blotchy type symptoms on leaves of inoculated Natsudaïdai seedling. E) Yellow blotch of inoculated Mexican lime seedling.

ly line patterns appeared systemically on leaves of many varieties of citrus seedlings such as Mexican lime (fig. 1E), Rangpur lime, Natsudaïdai (fig. 1C,D), Valencia orange, Madam Vinous sweet orange, Fukuhara sweet orange, Ponkan mandarin, and trifoliate orange. These symptoms also persisted after leaf maturation. The symptom in Marsh grapefruit seedlings was leaf flecking which was somewhat different from that in other varieties. Crinkling of leaves also appeared occasionally on Ponkan and satsuma mandarin seedlings. Some shoot dieback was found on some infected seedlings one year after leaf symptoms appeared. No symptoms were found on Eureka lemon seedlings.

Leaf symptoms such as vein

clearing, yellowing, blotchy patterns and ring spots which appeared on some citrus seedlings were quite similar to those of citrus ringspot reported elsewhere from the world (2, 4, 6, 8, 13, 14). However, there were some differences of shoot reaction between the present disease and citrus ringspot, namely, the former occasionally showed some dieback of shoots about one year after inoculation and no bark lesions, while the later showed dieback as a shock symptom and caused bark lesions on branches (5, 9, 10).

Mechanical inoculation. No sap transmission was obtained from affected citrus to many kinds of herbaceous plants such as *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Reynier, *Spinacia*

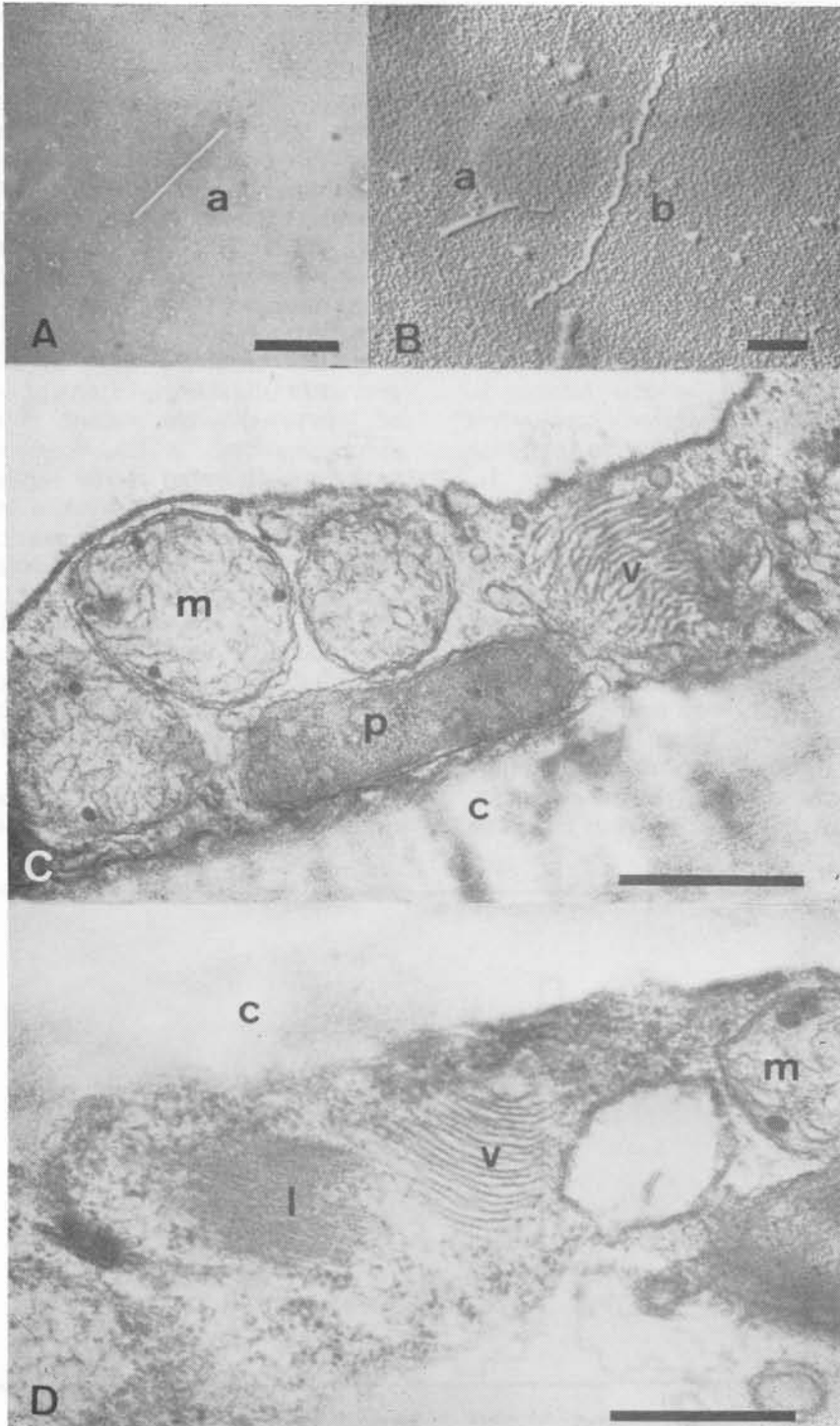


Fig. 2. Electron micrograph of virus particles (A and B) and leaf cells infected with citrus yellow mottle (C and D). a = citrus yellow mottle virus; b = citrus tristeza virus; c = cell wall; I = inclusion consisting of filaments; m = mitochondria; p = plastid; v = virus-like particles. Bar = 500 nm.

oleracea L. 'Minstar', *Beta vulgaris* L. 'Detroit Dark Red', *Vigna sinensis* Savi ex Haask. 'Kurodane-sanjaku', *V. unguiculata* (L.) Walp. 'Black eye', *Vicia faba* L., *Glycine max* Merr. 'Okuhara-wase', *Pisum sativum* L. 'Kinusaya', *Crotalaria spectabilis* Roth, *Nicotiana tabacum* L. 'Xanthi', *N. glutinosa* L., *Lycopersicon esculentum* Mill. 'Ponderosa', *Solanum melongena* L. 'Tsubakuro', *Physalis floridana* Rydb., *Capsicum annuum* L. 'California Univ.', *Cucumis sativus* L. 'Hatsukari', *Brassica campestris* L. 'Kokabu', *Tetragonia expansa* Murr., *Sesamun indicum* L., *Gomphrena globosa* L., and *Cantharanthus roseus* G. Don. Sap inoculation from the affected citrus trees to many kinds of citrus seedlings such as satsuma mandarin, Mexican lime, Ponkan, Eureka lemon, Marsh grapefruit, Natsudaidai, and Fukuhara orange was also unsuccessful. These results also differ from those of citrus ring-spot whose causal agent is transmitted mechanically from citrus to

citrus and to herbaceous plants (4, 5, 6, 11, 12).

Electron microscopy. Rod-shaped and slightly flexible particles were found in the partially purified preparations from young Fukuhara sweet orange leaves showing yellow mottle symptoms (fig. 2A,B). The same particles were observed in a preparation from diseased young leaves by the leaf-dip method. Aggregates of virus-like particles were also observed in ultrathin sections of cells of leaves showing yellow mottle symptoms (fig. 2C,D). According to the measurement on 159 particles in preparations made by the leaf-dip method, the size of the particles was mostly 690-740 nm in length with a range of 240-2,200 nm (fig. 3), and 12-14 nm in width. A few CTV particles were sometimes observed in the same samples. However, the morphology of these two virus particles were quite different (fig. 2B).

Back inoculation to Fukuhara sweet orange seedlings by stem-cut

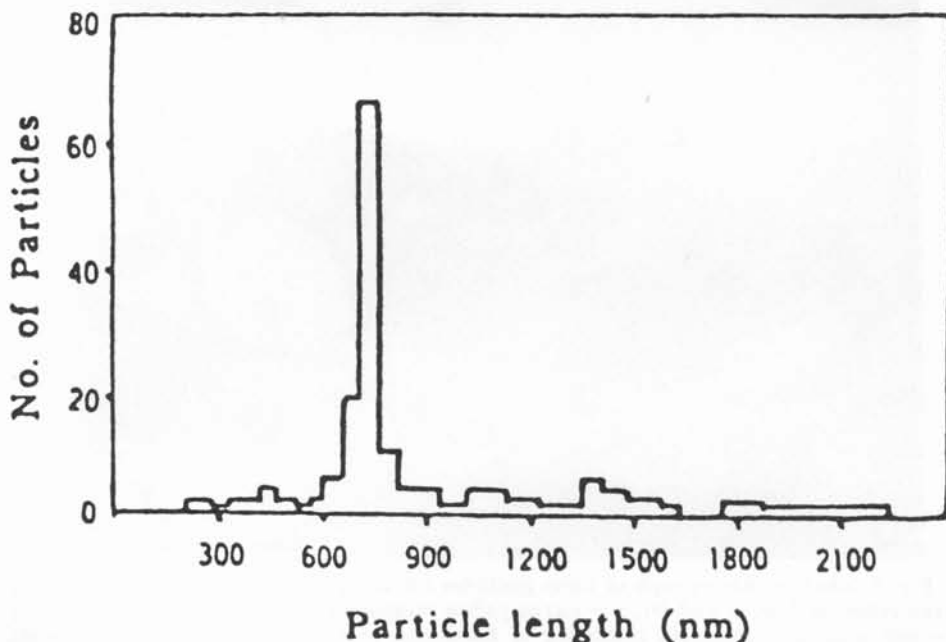


Fig. 3. Particle length of citrus yellow mottle virus measured from preparations made by the leaf-dip method.

inoculation. Three of six seedlings inoculated on October, 1979 (trial 1) and three of five seedlings inoculated on May, 1980 (trial 2) showed leaf symptoms by May, 1981. Symptoms appeared on leaves of shoots originating above or between the portions of cut-inoculated stem. These were vein clearing, yellowish halos, chlorotic spots or distinct yellow blotches and were similar to those of the seedlings graft-inoculated with original affected tissues. The rod-shaped virus-like particles were also detected from these infected leaves by the leaf-dip method.

Mexican lime seedlings which were graft-inoculated with the tissue of these infected Fukuhara sweet orange seedlings showed the same symptoms as the inoculum source seedlings about one year after inoculation. On the other

hand, CTV symptoms such as vein clearing and stem pitting never appeared in the seedlings of Mexican lime or Fukuhara sweet orange. ELISA tests also indicated that these seedlings were free of CTV.

These results indicate that the rod-shaped particle is the causal agent of the disease but is not related to CTV. Therefore, it is proposed that the new virus disease be named citrus yellow mottle and the causal agent citrus yellow mottle virus (CYMV).

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