

## Susceptibility of Trifoliolate Orange and Some Other Citrus Varieties to Satsuma Dwarf Virus

TSUNEKUNI MIYAKAWA

A GRAFT TRANSMISSIBLE disease of Satsuma orange (*Citrus unshiu* Marc.) was first reported and described by Yamada and Sawamura (5) as Satsuma dwarf disease. The characteristic symptoms of the disease are dwarfing of trees, small spoon-shaped leaves, and psorosis-like leaf patterns on young leaves (2). Tanaka *et al.* (3, 4) reported transmission of the virus from citrus to certain herbaceous plants by mechanical inoculation, but they could not return it to citrus. This paper describes experiments at the Tokushima Horticultural Experiment Station in 1965 and 1966 to determine the reaction of *Poncirus trifoliata* (L.) Raf. and certain other virus-indicator plants to the virus associated with the Satsuma dwarf disease.

### *Materials and Methods*

Virus-infected plant material was collected from six different areas of Japan where Satsuma dwarf disease is common, and one source was obtained from Dr. Yamada, Horticultural Research Station, Okitsu.

Tristeza virus was present in all inoculum sources. Consequently, the inocula obtained from the above sources were worked on *P. trifoliata* seedlings to free the Satsuma dwarf virus from tristeza. Thus, inocula containing Satsuma dwarf virus, but not tristeza, were used in these tests.

Seedlings were grown in 13 cm diameter clay pots in a greenhouse under insect-free conditions. Mites, leaf miners, and other insects were controlled by sprays. Greenhouse temperatures usually ranged between 20 and 30°C, but occasionally descended to 17°C on the coldest winter nights. Maximum temperatures sometimes reached 35°C on the hottest summer days.

Inoculations were made by inserting buds from the several sources of inoculum into T-slits in the bark of healthy test plants. To stimulate new growth, the seedlings were usually cut back to a single stem 15 to 18 cm high and were periodically inspected for symptoms.

### Results

SUSCEPTIBILITY OF *P. trifoliata* TO SATSUMA DWARF VIRUS.—Seedlings of trifoliolate orange inoculated with all seven sources developed a clear mottling, crinkling, and occasional twisting on young leaves of new growth flushes (Fig. 1,A,B). Symptoms usually developed 4 to 6 weeks after inoculation and were clearest and most severe on leaves that appeared after inoculation. The mottle disappeared as the leaves matured, and was not clearly visible in subsequent growth. Mature leaves of the infected seedlings usually appeared normal, but downward bending was observed occasionally.

To test further the susceptibility of *P. trifoliata* to the virus, two experiments were conducted in 1965 and 1966, using two virus sources, one from Dr. Yamada, the other from Ehime Prefecture. In the 1965 test, inocula from Satsuma dwarf-affected Satsuma orange trees and *P. trifoliata* seedlings previously infected with the original Satsuma dwarf buds were inoculated onto 4 *P. trifoliata* roots budded with healthy Satsuma tops. Healthy Satsuma orange was budded on trifoliolate orange, as a check. Characteristic symptoms of Satsuma dwarf disease appeared on the first flushes of growth from the Satsuma tops on all the trifoliolate roots 7 months after inoculation; growth from Satsuma buds on the same number of non-inoculated rootstocks showed no symptom in the same period (Fig. 1,D). In the 1966 test, 4 Satsuma orange seedlings were inoculated with the buds of *P. trifoliata* previously infected with the virus obtained from another *P. trifoliata* seedling. The same number of Satsuma seedlings remained non-inoculated, as checks. In this test, characteristic symptoms of the disease also developed on the new growth flushes of all the inoculated seedlings 3 months after inoculation, whereas the non-inoculated seedlings showed normal and healthy growth. These tests indicate that the seedlings of trifoliolate orange carried the virus.

SYMPTOMS ON VARIOUS CITRUS SEEDLINGS.—Host range tests were conducted in 1966, using inoculum from the 7 sources. As mentioned previously, these sources had been freed of tristeza virus. Three or 4 seedlings of each variety were used in the host range tests. The symptoms that developed are as follows.

*Poncirus trifoliata*.—Budwood from all 7 sources produced a clear

mottling and crinkling of the young leaves and sometimes twisting of the leaves (Fig. 1,A). Buds from infected trifoliolate seedlings reproduced the symptoms on the 4 trifoliolate plants and on the same number of Satsuma orange tops. When the same inoculum sources were worked on Mexican lime [*C. aurantifolia* (Christm.) Swing.] seedlings, none of the reactions typical of tristeza infection occurred even 1 year after inoculation. These results indicate the inoculum sources infected with the virus of Satsuma dwarf had been freed of the tristeza virus.

*Satsuma orange*.—A clear mottle sometimes resembling the oak-leaf pattern of psorosis appeared on the earlier flushes of new growth 4 to 5 weeks after inoculation. Leaf cupping or downward bending, typical of Satsuma dwarf infection, developed during the ensuing 2 to 3 months.

*Troyer, Morton, and Carrizo citrange* [*C. sinensis* (L.) Osb. x *P. trifoliata* (L.) Raf.].—A slight, but non-persistent mottle and crinkle developed on the young leaves of seedlings of these varieties 4 to 5 weeks after inoculation. Of the three citranges used, symptoms were clearest on Carrizo (Fig. 1,C).

*Rough lemon* [*C. limon* (L.) Burm. f.].—Conspicuous chlorotic spots developed on immature leaves of the new growth about 6 weeks after inoculation (Fig. 1,F). These persisted for a few weeks, then disappeared as the leaves matured. New shoots that developed subsequently were symptomless, and the seedlings continued to grow without any detectable symptom. Nevertheless, this host was one of the more sensitive and reliable indicator plants tested.

*Sweet orange* [*C. sinensis* (L.) Osb.].—A fleck-like mottle on the top leaves and also a slight circular or oak-leaf mottle on the immature leaves appeared occasionally, but disappeared when the leaves reached maturity.

*Calamondin* [*C. reticulata* var. *austera* x *Fortunella* sp.].—A clear mottle accompanied by severe crinkle developed on the leaves of new growth. These symptoms usually persisted and sometimes also appeared in subsequent growth.

*C. excelsa* WESTER.—A fairly clear mottle and slight crinkle developed on young leaves that were formed 3 to 4 weeks after inoculation and disappeared as the leaves matured. Subsequent growth was symptomless.

*Rangpur lime* (*C. reticulata* var. *austera* hyb.).—A clear, circular mottle developed on the immature leaves, but disappeared later.

*Mexican lime*.—A slight, non-persistent mottle, different from tristeza symptoms, developed on the young leaves 4 to 5 weeks after inoculation.

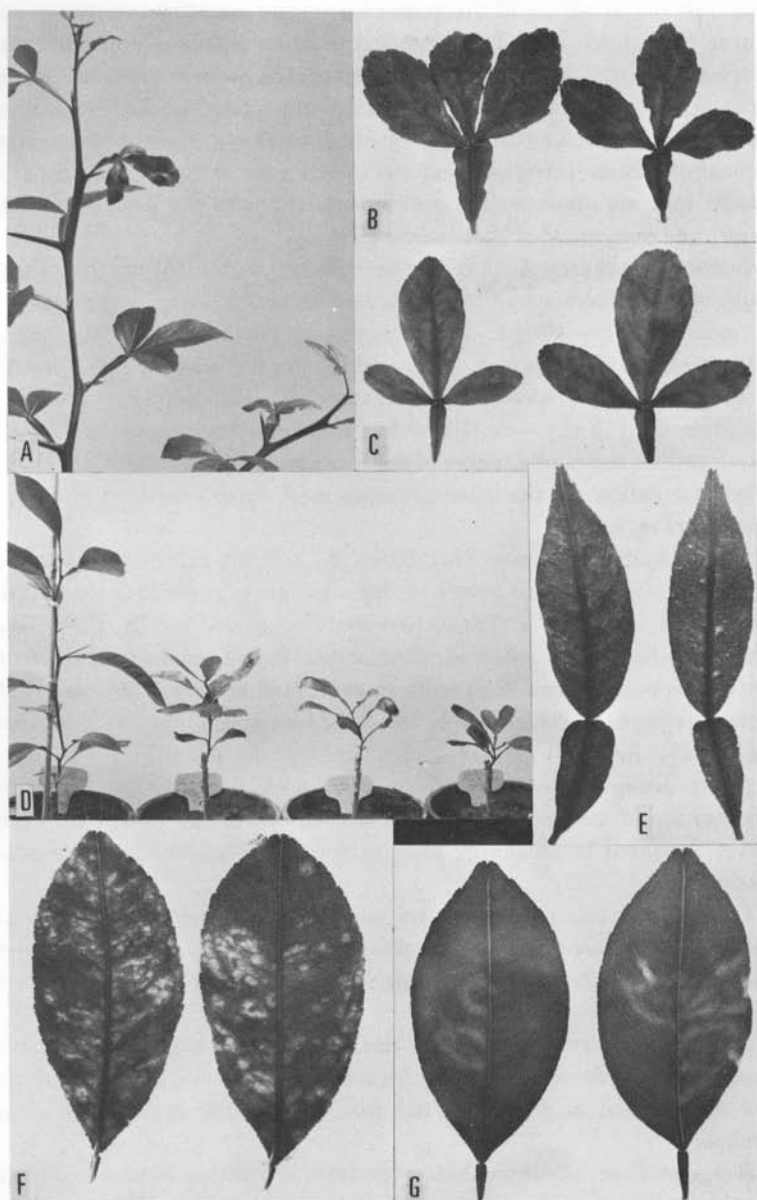


FIGURE 1. Symptoms of Satsuma dwarf virus. A. Twisting and crinkling of the young leaves of *P. trifoliata*. B. Young leaves showing mottle and irregular shape. C. Mottle on young leaves of Carrizo citrange seedling. D. Satsuma tops on tri-

*Other citrus.*—A mottle resembling the oak-leaf pattern of psorosis was observed on young leaves of Cleopatra mandarin (*C. reticulata* Blanco), Eureka lemon [*C. limon* (L.) Burm. f.], sour orange (*C. aurantium* L.), Yuzu (*C. junos* Sieb.), *C. depressa* Hayata, Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.], Clementine tangerine (*C. reticulata* Blanco), Murcott (*C. reticulata* hyb.), and Sampson, Thornton, and Wekiwa tangelo (*C. reticulata* Blanco x *C. paradisi* Macf.). This usually disappeared as the leaves matured. The varieties *C. depressa* (Fig. 1,G), Palestine sweet lime, Sampson tangelo, and Clementine tangerine showed other types of mottle and sometimes crinkle on the more advanced immature leaves. Seedlings of Orlando tangelo (*C. reticulata* x *C. paradisi*) and Marumera (*C. obovoidea* Takahashi) showed no detectable symptoms.

#### Discussion and Conclusions

Yamada and Sawamura (5) tested 34 citrus and related varieties and found six susceptible to Satsuma dwarf virus. Early reports (2, 5) of Satsuma dwarf virus emphasized the symptoms that appear on Satsuma orange, i.e., reduced size, bending and spoon-shaped appearance of the leaves, and dwarfing of trees. Flecking and oak-leaf zonation on young leaves were also described. However, tristeza virus is almost always present in the Satsuma budwood as we found in our index tests. This suggests that Yamada and Sawamura probably worked with a mixture of Satsuma dwarf virus and the tristeza virus and that the symptoms they reported may have been caused by that mixture.

We believe that passing the inoculum through *P. trifoliata* seedlings eliminated the tristeza virus. As a result, certain varieties that are susceptible to tristeza virus showed no symptoms of tristeza in our tests. The seven sources of Satsuma dwarf virus used in these tests produced similar symptoms on seedlings of *P. trifoliata*, whereas none of the other inoculum sources, those from Hassaku dwarf, from Wase-Satsuma showing Satsuma dwarf-like symptoms, and from several apparently healthy Satsuma orange trees, showed any of those symptoms. Slight variation in the severity of symptoms was observed in seedlings of *P. trifoliata* and certain other varieties inoculated with various sources of the Satsuma dwarf

---

*foliate roots showing the typical symptoms of Satsuma dwarf. Left: Non-inoculated check inoculated by the buds of P. trifoliata infected with the virus (freed of tristeza virus). E. Symptoms on young leaves of Yuzu seedlings that resemble the oak-leaf pattern. F. Clear chlorotic spots on immature leaves of Rough lemon. G. Mottle pattern on immature leaves of C. depressa.*

virus, but no evidence was found that Satsuma dwarf virus exists as different strains or consists of more than one virus. Inoculum from inoculated *P. trifoliata* seedlings always reproduced the same symptoms on the trifoliata seedlings and also infected the Satsuma orange tops. This suggests that *P. trifoliata*, a common rootstock of Satsuma orange in Japan, is susceptible to the Satsuma dwarf virus. The influence of tristeza virus on the symptoms of Satsuma dwarf was not determined in our tests.

The symptoms which appear on various citrus varieties are difficult to group definitely but may be described as follows: 1) The most common symptom is mottle or fleck-like mottle that appears on the young leaves of Cleopatra mandarin, sweet orange, Eureka lemon, sour orange, Yuzu (Fig. 1,E), Satsuma, Sampson, and Wekiwa tangelo. This symptom resembled a psorosis-type pattern on young leaves, but did not accompany crinkle. The typical leaf pattern of psorosis was not observed. 2) Another common but somewhat different mottle (Fig. 1,B,C) developed on more advanced new growth and was sometimes accompanied by crinkle. The symptom of Satsuma dwarf virus on seedlings of *P. trifoliata*, Troyer, Morton, and Carrizo citrange, *C. excelsa*, and *C. depressa* are of this type. Mottled on sweet orange, Sampson tangelo, and Palestine sweet lime are also similar to this, but usually did not accompany crinkle. 3) Finally, chlorotic spots were produced on the immature leaves of Rough lemon (var. South Africa) seedlings (Fig. 1,F) which appear similar to those reported by Fraser (1) on the leaves of Eureka lemon seedlings inoculated with lemon crinkly-leaf virus. No other species or variety of those tested produced this type of symptom, but the larger circular mottle on Rangpur lime was somewhat similar. Downward bending, or cupping of leaves and stunting of shoot growth (Fig. 1,D) was uncommon except on Satsuma and on a few *P. trifoliata* seedlings.

In our tests, almost all of the citrus and relative varieties showed susceptibility and symptoms. These results indicate that the virus of Satsuma dwarf disease has a very wide range of hosts in citrus and related plants.

ACKNOWLEDGMENTS.—The author wishes to thank R. M. Endo, University of California, for critical reading of the manuscript, and J. M. Wallace and R. J. Drake for seeds of certain citrus varieties and for suggestions.

#### *Literature Cited*

1. FRASER, L. R. 1961. Lemon crinkly leaf virus, p. 205-210. In W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.

2. TANAKA, S., and YAMADA, S. 1961. Citrus virus diseases in Japan, p. 247-252. In W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
  3. TANAKA, S., and KISHI, K. 1963. Studies on indicator plants for citrus viruses. I. Mechanical inoculation on leguminous plants with sap from Satsuma dwarf tree. Ann. Phytopath. Soc. Japan 28(5): 262-267.
  4. TANAKA, S., KISHI, K., and YAMADA, S. 1965. Researches on the indicator plants of Satsuma dwarf and Hassaku dwarf disease, p. 260-267. In W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
  5. YAMADA, S., and SAWAMURA, K. 1952. Studies on the dwarf disease of satsuma orange, *Citrus unshiu* Marcovitch (Preliminary report). Hort. Div., Tokai-Kinki Agr. Expt. Sta. Bull. 1: 61-71.
-