

# Occurrence of Satsuma Dwarf Virus in Zhejiang Province, China

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## ABSTRACT

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This is the first report of the occurrence, damage, and host range of satsuma dwarf virus (SDV) in China. Observations of field trees and transmission tests done from 1987 to 1989 in Zhejiang Province with indexed white sesame (*Sesamum indicum*), cowpea (*Vigna unguiculata*), and satsuma mandarin (*Citrus unshiu*) resulted in development of symptoms typical for SDV. Back transmission tests by sap inoculation from infected white sesame to white sesame were positive. Twelve satsuma variant cultivars commonly grown in Zhejiang Province and four major cultivars—Ben-di-zao (*C. succosa*), Zhao-ju (*C. subcompressa*), Man-ju (*C. tardiferax*), and Ponkan (*C. poonensis*)—were found infected with SDV. All satsuma mandarin field trees exhibited some infection when critically examined for symptoms associated with SDV. It is probable that SDV is now widespread in Zhejiang Province and has been present in China for a long time.

The "satsuma dwarf" disease has been known in Shizuoka Prefecture, Japan, since the early 1930s. Yamada and Sawamura (13) reported transmission from diseased field trees to healthy citrus plants by graft-inoculation and concluded that a virus was responsible for this disorder. They reported that it was a new disease which was spreading and was symptomatically different from psorosis, tristeza, stubborn, and other known virus diseases of citrus. They proposed the name "dwarf disease of satsuma orange," which was later shortened to "satsuma dwarf." They reported that infected trees showed inhibition of growth of new twigs with multiple sprouting, shortened internodes, and abnormal boat- or spoon-shaped leaves. These diagnostic field symptoms were associated with the dwarfed and stunted appearance of the tree.

Studies on graft transmission, mechanical inoculation, host range, and properties of the causal virus have been reported (1,4,5,7,9-11) and reviewed (12). The disease is widespread in Japan, damaging not only satsumas but also other mandarins, *Citrus natsudaidai* Hay, and navel oranges (2,7,10). Citrus mosaic virus, natsudaidai dwarf virus, and navel orange infectious mottling virus reported from Japan are apparently related to the satsuma dwarf virus (SDV) (2,10). Azeri (1) reported wide distribution of SDV in the Aegean Coast of Turkey. The disease has not been reported from other citrus-producing countries.

Observations of certain field symptoms made by P. F. Cui in 1986 in the Huangyan district of Zhejiang Province, China, suggested the possible presence of satsuma dwarf disease. Field observations and transmission experiments were begun to ascertain the presence of SDV. This paper reports the results of those experiments.

## MATERIALS AND METHODS

**Observation and examination of satsuma trees in the field.** Five- to 20-year-old trees of satsuma mandarin (*C. unshiu* Markovich) on trifoliolate orange rootstock were visually examined for symptoms of spoon- or boat-shaped leaves in the spring flush of growth. Five trees of each of 12 varieties of satsuma were selected at random for examination. All leaves on 10 shoots from the inner and lower section of each tree were rated for their boat- or spoon-shaped appearance and any malformation as in Figure 1A. In addition, trees were examined for the presence of witches'-broom and shortened internodes associated with the disease as in Figure 1B.

**Assay to index plants—bud transmission.** Bud-graft transmissions were made from symptomatic branches of field trees of satsuma mandarin found in the Huangyan district to satsuma mandarin seedlings. All seedlings used for indexing were grown in steam-sterilized soil and maintained in an insect-proof screenhouse. Pests were carefully controlled by periodic pesticide sprays. All test tools were disinfected with a 1% sodium hypochlorite solution (8). Inoculation was by two bud grafts to each of seven seedlings, and the seedlings were cut back at 20–25 cm above the soil surface at the time of inoculation. Inoculated plants were kept at 20–22 C in a controlled temperature

chamber in the laboratory. Wrapping tapes were cut 3 wk after inoculation. Uninoculated seedlings were used as controls.

**Assay to index plants—mechanical transmission.** Mechanical transmissions were made by sap inoculation from young symptomatic shoots from field trees to seedlings of white sesame (*Sesamum indicum* L.) and cowpea (*Vigna unguiculata* (L.) Walp.) The young shoots were obtained from satsuma mandarin cvs. Ben-di-Zao (*C. succosa* Hort. ex Tan.), Man-ju (*C. tardiferax* Hort. ex Tan.), Zhao-ju (*C. subcompressa* Tan.), and Ponkan (*C. poonensis* Hort.). Districts from which shoots were obtained were Taizhou, Wenzhou, and Ningbo, all in Zhejiang Province. Approximately 10 cm of the soft young shoots from affected trees were collected, placed in plastic bags, and put in an ice chest for transportation to the laboratory. They were held under refrigeration until used. Young leaves were macerated in a mortar with a 1:5 (v/v) 1/15 M Sorensen's phosphate buffer solution at pH 6.98. Leaves of the indicator plants were first dusted with 600-mesh Carborundum and then rubbed with a small piece of absorbent cotton dipped in the inoculum. After inoculation, plants were rinsed with tap water. Control plants were similarly dusted and rubbed with the same buffer solution and rinsed with tap water. Inoculated plants of white sesame were kept at 20–22 C for 12 hr in the laboratory, after which they were removed to the screenhouse. All inoculated plants of cowpea were held continually in the laboratory at 20–22 C.

Serial transmission tests were done by sap inoculation from symptomatic white sesame plants to white sesame seedlings by sap inoculation in the same manner as described above.

## RESULTS

**Observation and examination of satsuma mandarins in the field.** Leaves and shoots from all trees selected at random showed typical symptoms of boat- or spoon-shaped leaves, downward leaf curling, shortened internodes, and witches'-broom of twigs (Fig. 1A,B). These symptoms were observed to various degrees on all of the shoots examined. Although the severe symptoms described above were not observed on varieties other than satsuma, mild symptoms were apparent.



**Fig. 1.** (A) Individual leaves showing characteristic boat or spoon shapes, which are symptomatic for satsuma dwarf disease. These were found in the field on most satsuma mandarin trees growing in Zhejiang Province, China. Normal leaves are on the right. (B) Shoots from trees selected at random showing typical symptoms of compressed growth, witches'-broom, downward leaf curling, and boat- or spoon-shaped leaves.

**Graft transmission tests.** After 7 wk at temperatures of 20–22 C, young leaves on five of the seven graft-inoculated seedlings showed the boat- or spoon-shaped leaves diagnostic for SDV (2). Comparisons made with the uninoculated control plants showed definitive positive infection in graft-inoculated plants.

**Mechanical transmission.** The number of sesame plants found infected after sap-inoculation from young shoots of the various satsuma and other cultivars are given in Table 1. Leaves of the inoculated white sesame plants developed necrotic local lesions after 10 days. The systemically infected new growths developed curling and were malformed. In later stages, the tip leaves became necrotic and dieback proceeded down the stem. Shock symptoms were apparent at temperatures of 20–22 C. Tissue taken from all 12 satsuma varieties and the four major cultivars indexed positive for SDV. Also, tissue from two satsuma trees taken from the Wenzhou, Taizhou, and Ningbo districts indexed positive for SDV. All six serial transmissions from infected white sesame to white sesame index plants were positive.

When cowpea was used as a host, 10 of 35 cowpea plants showed necrotic streaks on the petioles and stems in the laboratory at 20–22 C a few days after

inoculation. Systemically infected upper leaves did not grow, and infected plants showed poor growth. Systemically infected upper leaves of all symptomatic cowpea plants died in 5–7 days.

## DISCUSSION

This is the first report of indexing for satsuma dwarf virus in China and shows that the disease is serious and widespread in commercial orchards in three districts in Zhejiang Province. The disease was present in all of the 12 selections of satsuma and in four other major commercial cultivars of mandarin commonly grown in Zhejiang Province. It appears that the distribution of SDV is widespread. The full extent of infection awaits further testing with serology (6,15) or by monoclonal antibodies (15). The use of virus-free budwood and certification will be recommended to prevent distribution of infected material in propagative budwood.

Mandarins have been grown in Zhejiang province for over 1,400 yr and many legends are connected with its origin (14). Satsuma mandarins now growing in Zhejiang Province were imported from Japan some 70 yr ago. However, the satsuma growing in Japan is believed to have originated as a chance seedling from mandarin cultivars imported into Japan

**Table 1.** Sap transmission of SDV from symptomatic shoots of field trees to six white sesame plants

Cultivar <sup>a</sup>	Infected plants/ surviving plants
Satsuma	
Miyagawa	6/6
Tachimina	3/4
Hatsuyama	4/4
Okitsu	3/4
Hankan No. 20	3/3
Nankan No. 4	6/6
Rin	3/3
Yonezawa	2/3
Koyama	2/2
Shoda	6/6
Hannoa	6/6
Ishikawa	1/2
Miyagawa <sup>b</sup>	3/4
Miyagawa <sup>c</sup>	2/3
Mandarin	
Ben-di-zao	2/4
Man-ju	2/6
Ponkan	2/6
Zhaoju	2/5

<sup>a</sup> All from the Taizhou District, Zhejiang Province.

<sup>b</sup> From the Ningbo District, Zhejiang Province.

<sup>c</sup> From the Wenzhou District, Zhejiang Province.

from Huangyan, Wenzhou District, Zhejiang Province, China, some 500 yr ago (3). It is possible that the satsuma dwarf disease has been present in China since very early times.

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