China Laurestine: A Symptomless Carrier of Satsuma Dwarf Virus which Accelerates Natural Transmission in the Fields

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ABSTRACT. Natural infection of satsuma mandarin by satsuma dwarf virus (SDV) occurred in a field where China laurestine, *Viburnum odoratissimum* Ker., was planted as windbreaks. A high percentage of the windbreak plants were infected. Virus-free nursery trees of China laurestine became infected with SDV 2-8 months after planting in potted soil collected from SDV-infested fields. Infected trees were free of symptoms. These results indicate that SDV-transmission is enhanced by the presence of China laurestine and that it may be an important inoculum source for citrus trees.
Index words. soil transmission, ELISA.

Satsuma dwarf (SDV) was proven to be a graft-transmissible virus disease in 1952 (11), but had been noticed in Japan for about 20 years previously as an unknown disorder. The natural spread of SDV in the field has been noted since its discovery. Izawa (4) investigated several affected fields and reported that the disease was present in only one tree in 1933, and extended to 48 trees by 1953, and to 153 trees by 1965. Disease spread was in a concentrated circle, and trees replanted after removal of the original diseased trees became infected within several years. Based on these observations SDV has been considered soil-transmissible. Trials to reproduce soil-transmission in pot tests and to find the vector have been unsuccessful.

Recently, one of the authors observed rapid spread of SDV in a field where China laurestine, *Viburnum odoratissimum* Ker. (syn. *V. awabuki* K. Koch), was planted as a windbreak (7). These windbreak plants indexed positively for SDV by ELISA. This paper reports the presence of SDV in China laurestine and the rapid acquisition of SDV by China laurestine trees from infested soil.

MATERIALS AND METHODS

Indexing. China laurestine and satsuma mandarin trees were indexed for SDV by ELISA (1) using anti-SDV serum. Tender, immature shoots of citrus plants were macerated with 10 volumes (V/W) of phosphate-buffered saline which contained 0.05% Tween-20 (PBS-tween) plus 2% polyvinylpyrrolidone (PVP) and 0.05% 2-mercaptoacetic acid.

Immature leaves of China laurestine and rootlets of these plants and of trifoliate orange rootstock, were macerated in PBS-Tween plus 2% PVP and 0.05% L-cysteine. A microplate filled with sample extracts was incubated at 28 C for two h and then transferred to a refrigerator overnight. Alkaline phosphatase-antibody conjugates were diluted with PBS-tween plus 0.1% bovine serum albumin, which prevented nonspecific reactions. Incubation conditions for coating plates and application of conjugates was 28 C for 4 h. Other procedures were according to Clark and Adams (1).

Back inoculation. Two-week-old plants of white sesame were mechanically inoculated with extracts from the SDV-ELISA-positive China laurestine. Inoculated plants were kept at 22 C in a greenhouse. After development of typical symptoms, mechanical transmission of SDV from diseased sesame to Etrog citron seedlings were made by slashing the receptor plant 60 times with a knife contaminated with sap from sesame. Five to six months after inoculation the citrons were assayed by budding to a virus-free rough lemon rootstock top-grafted with virus-free satsuma mandarin.

Acquisition of SDV by China laurestine from soil. China laurestine trees proved SDV-free by ELISA were propagated as rooted cuttings in autoclaved soil. Propagated plants were potted in soil collected from the 5-10 cm deep zones of the rhizosphere under SDV-affected satsuma mandarin trees.

These plants were placed outdoors at the research station for five months and then transferred into a glasshouse. New sprouts were periodically indexed for SDV by ELISA.

RESULTS

Disease occurrence in the field. High incidence of SDV was found in a new plantation at Kanayama, Kumano City, Mie Prefecture, Japan. The field was planted in 1965-70 after cutting through a hill of about 120 ha of former forest land. Early satsuma mandarin trees on trifoliate orange rootstock were planted from distant nurseries. Trees were spaced 2.0 x 1.8 m. China laurestine trees were planted at the same time as a windbreak in rows 25 m apart.

Symptoms of satsuma dwarf were found around 1975 on a few satsuma trees. Since that time affected trees have increased markedly, especially in an area adjacent to the windbreak (fig. 1). Disease spread occurred along the row of windbreaks, and trees near the windbreaks showed severe symptoms. By 1982, 906 satsuma trees (about two ha of 95 ha) were affected. The windbreak plants were free of virus-like symptoms. ELISA revealed that the symptomatic trees of satsuma and the windbreak plants adjacent to them were carrying SDV. In contrast, windbreak plants away from the affected satsuma were mostly free of SDV.

Detection of SDV from China laurestine. Tender and immature leaves and rootlets were collected from infected China laurestine trees at Kanayama. Similar tissues of infected satsuma mandarin trees and of their trifoliate orange rootstocks were also collected from Okabe, Shizuoka Prefecture. Rootlets of trifoliate orange were divided into three groups, whitish and tender,

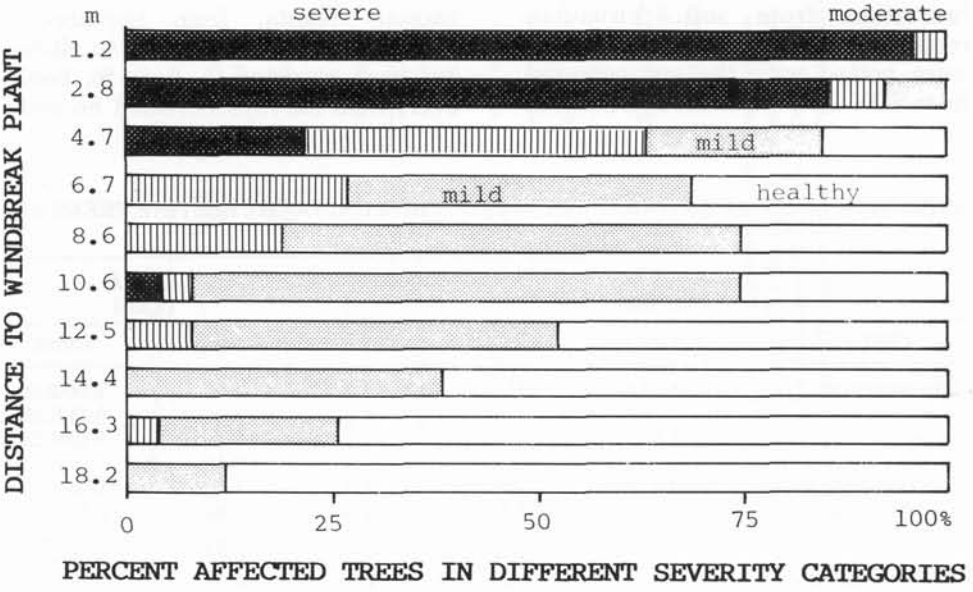

Fig. 1. Relation between severity of satsuma dwarf in an early satsuma mandarin orchard and the distance to the China laurestine windbreak.

brownish but not yet hardened, and hardened. These samples were tested by ELISA.

ELISA results indicated that SDV content of immature leaves of affected China laurestine was as high as that of affected satsuma leaves (table 1). Whitish and tender rootlets of China laurestine had a virus concentration similar to that of trifoliate orange roots, but concentrations in both were less than those in the immature leaves. SDV content of immature brown rootlets and hardened roots was very low.

Sap inoculation to sesame plants using immature leaves and rootlets of the infected China laurestine resulted in production of typical local and systemic lesions. Mechanical transmission by knife-slashing from infected sesame to Etrog citron was successful in three out of eight seedlings inoculated (based on SDV-ELISA assays). Bud inoculations from SDV-ELISA-positive citrons caused typical boat- or spoon-shape leaf symptoms on satsuma mandarin. This confirms the presence of SDV in the China laurestine plants collected from the field at Kanayama.

Acquisition of SDV by China laurestine from soil. Virus-free rooted cuttings of China laurestine were potted with the soil collected from SDV-infested fields at Okabe, in July, 1985. ELISA tests of the plants showed that one of 56 trees became SDV-positive by mid-September, 1985, and that three more trees became positive by late March, 1986 (table 2). In contrast, no infection of SDV was detected in 238 seedlings of trifoliate orange within 2 yr after planting in the similar soil.

DISCUSSION

Field surveys at Kanayama and the experiments done show that the marked incidence of SDV in satsuma trees was associated with China laurestine trees which rapidly acquire SDV through the soil and then become important inoculum sources. As China laurestine trees are usually propagated by rooted cuttings and are widely used as a windbreak in Japan, SDV contamination in these plants should not be overlooked.

In early years, it was thought that SDV was transmitted not only by grafting, but also by a flatid planthopper, *Geisha distinctissima* Walker (12, 14). However, subsequent experiments showed no evidence for planthopper transmission (5, personal communication, T. Miyakawa). Mechanical transmission from citrus to herbaceous plants, from herbaceous plants to citrus, and citrus to citrus has been reported (5, 6, 8, 9), however, field surveys revealed no con-

TABLE 1
DETECTION OF SATSUMA DWARF VIRUS (SDV) FROM CHINA LAURESTINE TREES BY ELISA

Sample				ELISA (A405)	
Plant	Location	Tissue[z]	No.	Average	Range
China laurestine	Kanayama	IL	2	2.00	2.00-2.00
—	—	IWR	3	0.64	0.44-0.89
—	Okitsu	IL	10	0.21	0.19-0.22
—	—	IWR	1	0.18	
Satsuma mandarin	Okabe	IL	8	1.90	1.66-2.00
Trifoliate orange	—	IWR	8	0.65	0.20-1.94
—	—	IBR	8	0.33	0.18-0.85
—	—	HR	8	0.36	0.19-0.81
Virus-free satsuma		IL	1	0.21	

[z]IL: immature leaves: IWR: immature and whitish rootlets; IBR: immature but brownish rootlets; HR: hardened rootlets.

TABLE 2
TRANSMISSION OF SATSUMA DWARF VIRUS (SDV) TO CHINA LAURESTINE THROUGH SOILS COLLECTED FROM DISEASE-INFESTED FIELDS

Soil used for potting	No. of trees planted[z]	No. of trees positive[y]	
		Sep. 1985	Mar. 1986
Miwa, Okabe—1	11	0	0
— —3	11	0	1
Muraryo, Okabe— 8	12	1	3
— —10	12	0	0
— —11	10	0	0
Autoclaved soil	15	0	0

[z]SDV-free nursery trees of China laurestine were planted in pots, July 15, 1985.
[y]SDV infection detected by ELISA.

tact transmission even where twigs of affected trees and healthy trees were touching (10). Based on many observations, mechanical transmission seems very rare in the field.

Transmission through pollen has not been studied, but the observed spread of the disease in a radial manner (4, 10) does not suggest pollen-transmission. Seed transmission was observed in sap-inoculated Kidney beans but not in citrus (5).

Izawa (4) reported that the virus transmission occurred across a path one meter wide and also across a soil bank 3 m high, but not over a ditch 50 cm wide and 50 cm deep with flowing water. Reinfection was observed in replant trees planted where affected trees had been removed 3 yr earlier. Similar transmissions have been observed in many other places (3, 10, 13), and suggest that satsuma dwarf is a soil-borne disease.

Miyakawa (personal communication) demonstrated that SDV was transmitted from citrus to citrus in mix-planted pots using soil collected from a SDV-infested field. No evidence of the virus transmission by *Xiphinema americanum* and *Tylenchulus semipenetrans* was observed. Imada and Tanaka (2) observed resting spores of what was probably *Olpidium* sp. in the rootlets of affected citrus rootstocks and in roots of some weeds near the tree. They tried to transmit SDV to tobacco plants through soil which contained the SDV-infected rootlets of trifoliate orange. Only one tobacco plant showed typical symptoms, but further confirmation of SDV-infection and reproduction of fungal transmission have been unsuccessful.

Many trials have been made to induce soil transmission of SDV to virus free trifoliate orange trees potted in soil collected from SDV-infested fields, without success. This experiment is the first demonstration of the soil transmission of SDV. China laurestine trees may acquire SDV rapidly through soil and it is easily propagated from cuttings or seed. Therefore, this plant should be very useful as a trap plant to discover the vector of SDV.

ACKNOWLEDGMENT

The authors are grateful to Dr. T. Miyakawa for his kind information, and also to Dr. S. M. Garnsey for his critical review of the manuscript.

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