

## Satsuma Dwarf

### Reaction of Some Citrus and Herbaceous Plants to Satsuma Dwarf Virus Strains

T. MIYAKAWA

SATSUMA DWARF disease was first described as a virus disease by Yamada and Sawamura in 1952 (7). They reported that out of 35 citrus species and hybrids tested, 5 were susceptible and developed symptoms. The disease was, at first, characterized by boat- or spoon-shaped leaves produced on satsuma (7). Recently, the virus associated with the disease has been mechanically transmitted from citrus to some herbaceous plants (1, 3, 6) and returned to citrus from the herbaceous plants (1). Miyakawa (4) reported that all 27 varieties of citrus or related plants tested became infected, but only satsuma had symptoms typical of satsuma dwarf.

Two other diseases resembling satsuma dwarf, but named natsudaidai dwarf and citrus mosaic disease, have been found in other districts of Japan. The causal viruses were also found to be sap-transmissible to some herbaceous plants (1).

This paper describes reactions of

some citrus and herbaceous plants to the virus strains associated with satsuma dwarf and related diseases. Similarities and differences in host reaction to the viruses or virus strains are discussed.

#### *Materials and Methods*

**VIRUS SOURCES.**—Satsuma dwarf and related virus isolates were collected from several parts of Japan. An isolate obtained from the Horticultural Research Station, Okitsu, Shizuoka (courtesy of Dr. Yamada) was used as the standard for satsuma dwarf. The virus isolates used are listed in Table 1. The isolates were graft-inoculated into trifoliolate orange seedlings growing in a greenhouse under insect-free conditions. Later, the infected trifoliolate orange buds, apparently not tristeza-reactive on West Indian lime, were used for inoculating citrus.

**INOCULATION OF CITRUS.**—Seedlings or budlings were planted in 13 cm diameter clay pots in a green-

house under insect-free conditions and inoculated by inserting infected buds into T-cuts. At least 3 seedlings of each species and variety were inoculated with each virus isolate. The seedlings were usually cut back to a single stem 15–18 cm high to stimulate new growth and were periodically inspected for symptoms. Most inoculated plants were

leaves used for inoculum were frozen and stored at  $-20^{\circ}\text{C}$  before use. The juice was gently rubbed on carborundum-dusted leaves, which were immediately rinsed with tap water.

### Results

REACTION OF COWPEA AND SESAME.  
—Cowpea seedlings (Wisconsin

TABLE 1. SATSUMA DWARF AND RELATED VIRUS ISOLATES USED IN THIS TEST

Virus isolate	Place of origin	Original host and symptoms	Other viruses present
SDV 1 (satsuma dwarf)	Shizuoka	<i>C. unshiu</i> , spoon- or boat-shaped leaves, dwarfing	Tristeza, but no exocortis, others not indexed
SDV 2	Ehime	"	Tristeza, others not indexed
SDV 3	Kanagawa	"	"
SDV 4	Aichi	"	"
SDV 5	Kumamoto	"	"
SDV 6	Oita	"	"
NDV (natsudaidai dwarf)	Yamaguchi	<i>C. natsudaidai</i> , dwarfing, variegation of leaves	"
CMV (citrus mosaic)	Wakayama	<i>C. unshiu</i> , mottle on fruits, milder leaf-bending similar to satsuma dwarf	"

grown and observed in the greenhouse for a year, and some were kept several years for continuing observations.

INOCULATION OF HERBACEOUS PLANTS.—Herbaceous plants to be inoculated were planted in 9 cm diameter clay pots in a greenhouse. Young leaves of citrus seedlings or budlings showing symptoms (in most cases infected satsuma leaves were used) were triturated in a mortar with 2 parts (w:v) of 0.05M  $\text{K}_2\text{HPO}_4$  solution. The affected

source, courtesy of Dr. S. M. Garnsey), inoculated at the primary leaf stage, developed chlorotic lesions on the inoculated leaves in about 2 weeks. Stem necrosis and general mottling of mature leaves appeared later (5). The results are summarized in Table 2. All satsuma dwarf virus (SDV) isolates, except SDV 6, easily infected cowpea seedlings and caused symptoms. SDV 6 and citrus mosaic virus (CMV) sometimes caused faint reactions, but natsudaidai dwarf (NDV) had no virulence

to cowpea plants in repeated tests.

On white sesame (courtesy of Dr. K. Kishi) all SDV, NDV, and CMV isolates caused reactions. SDV and CMV caused local lesions on the inoculated leaves and systemic necrosis on advanced leaves in 7–10 days (Table 3). NDV showed little virulence, and caused only a few lesions and slight necrosis on this host.

**SYMPTOMS ON SATSUMA.**—On seedlings or budlings of satsuma, all 8 isolates caused persistent cupping and downward bending of the leaves, typical of satsuma dwarf. NDV and CMV likewise produced satsuma

dwarf symptoms on the budlings. Basically these symptoms were the same type, but those caused by CMV were a little milder (Fig. 1).

**REACTIONS OF SOME OTHER CITRUS PLANTS.**—Kunembo seedlings were inoculated with SDV 1, 2, 6, NDV, and CMV. Ten months after bud-inoculation, seedlings infected with SDV 2 and 6 showed persistent upward bending on the mature leaves, and growth was somewhat retarded (Fig. 2). Other isolates used have shown no noticeable symptoms to date, so observations are being continued.

TABLE 2. REACTION OF COWPEA, *Vigna sinensis*, TO SATSUMA DWARF STRAINS

Virus isolate	Replication						Total
	1	2	3	4	5	6	
SDV 1	7/7 <sup>a</sup>	12/12	12/12	11/12	11/11	12/12	65/66
SDV 3						12/12	12/12
SDV 4						12/12	12/12
SDV 5						9/12	9/12
SDV 6		1/12	12/12		1/34		14/58
NDV	0/10	0/12	0/16	0/11			0/49
CMV	12/12	1/12	8/16	1/31			22/71
Check				0/8	0/8		0/16

a. Number of plants, infected/inoculated.

TABLE 3. REACTION OF SESAME, *Sesamum indicum*, TO SATSUMA DWARF VIRUS STRAINS

Virus isolate	1		2		3		Total	
	LI <sup>a</sup>	Sn <sup>b</sup>	LI	Sn	LI	Sn	LI	Sn
SDV 1	12/17 <sup>c</sup>	12/17	8/8	8/8			20/25	20/25
SDV 2			8/8	8/8			8/8	8/8
SDV 3					4/5	2/5	4/5	2/5
SDV 4					3/8	0/8	3/8	0/8
SDV 5					2/7	0/7	2/7	0/7
SDV 6			7/15	5/15	7/15	0/15	14/30	5/30
NDV	7/20	0/20	5/7	1/7	7/7	2/7	19/34	3/34
CMV	4/9	4/9			1/5	1/5	5/14	5/14
Check	0/7	0/7	0/8	0/8	0/6	0/6	0/21	0/21

a. LI, local lesion.

b. Sn, systemic necrosis.

c. Number of plants, infected/inoculated.

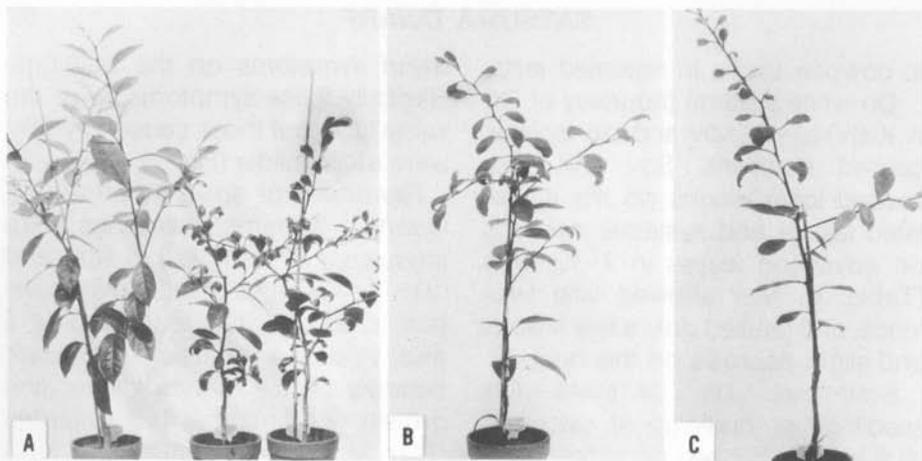


FIGURE 1. Symptoms of satsuma dwarf and related viruses on satsuma budlings: A. Non-inoculated (left), and infected with SDV 1 (right). B. Symptoms of NDV, similar to satsuma dwarf. C. Symptoms of CMV.



FIGURE 2. Symptoms in mature leaves of citrus seedlings infected with SDV 2. A. Crinkling of leaves of a tengu mikan seedling. B. Upward cupping and crinkling of leaves of an infected kunembo seedling.

Tengu mikan seedlings were inoculated with SDV 1, 2, 6, NDV, and CMV. Persistent crinkling of leaves developed from infection by all isolates except NDV, although some differences in symptom severity were evident.

Yatsushiro mikan plants were inoculated with 5 isolates, including NDV and CMV. All isolates except CMV caused dwarfing, yellowing, and small leaves, symptoms that persisted into the advanced stages of the disease, but no isolate caused symptoms characteristic of satsuma dwarf. The CMV isolate caused permanent crinkling of expanding leaves.

Ujukitsu appears to be monoembryonic, so its seedlings were not perfectly uniform. Symptoms were variable. Some seedlings developed mild or severe leaf-bending similar to that of satsuma dwarf. Grapefruit seedlings inoculated with SDV 1, 2, 4 or CMV reacted with a faint mottling on young leaves, but this symptom disappeared in a few months. SDV 6 was tested simultaneously, but no reaction was detected. On the other hand, NDV caused distinct, somewhat circular blotches on immature leaves (Fig. 3). These blotches persisted for several months, but gradually disappeared after variegated leaves appeared on subsequent growth.

Rough lemon seedlings were inoculated singly with NDV, CMV, and all SDV isolates. SDV 1, 2, 3, 4, and 5 caused mottling, spotting, and occasional line-pattern mottle in immature leaves in 6–8 weeks. However, SDV 6 seemed to be unstable, and caused a few faint spots on some seedlings.

Symptoms caused by CMV and NDV were also variable and less severe than those from most of the SDV isolates. However, large spots, sometimes accompanied by crinkling, were detected on the seedlings infected with NDV. These symptoms were not persistent.

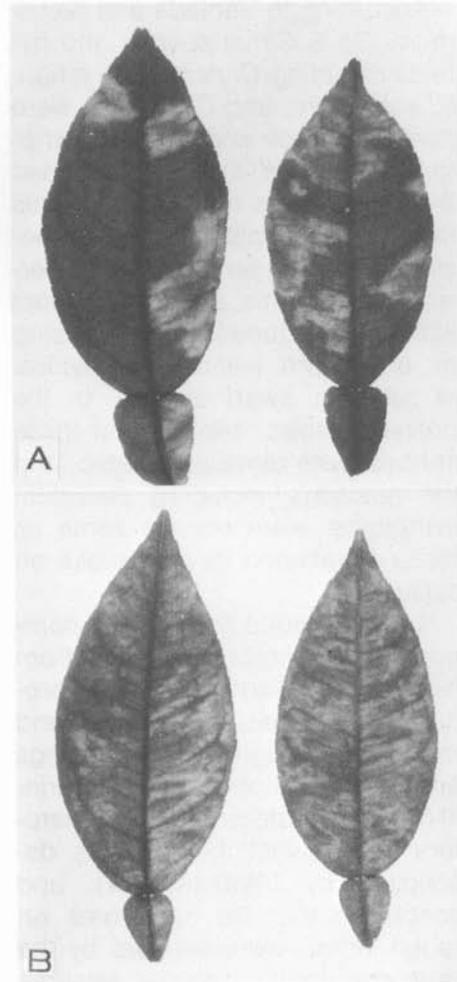


FIGURE 3. Mottling on immature leaves of grapefruit seedlings. A. Large spots induced by NDV. B. Irregular mottling induced by CMV.

Dweet tangor seedlings were inoculated with SDV 1, 2, 6, NDV, and CMV. The isolates, except SDV 6, caused clear flecking or line-pattern mottle on young leaves. SDV 2 caused the clearest and severest symptoms; SDV 6 caused no detectable reaction.

### Discussion

According to Yamada and Sawamura (7), 5 *Citrus* species and hybrids including *C. nobilis*, *C. tengu*, *C. yatsushiro*, and *C. ujukitsu* were infected by SDV and showed symptoms. Miyakawa (4) recently reported that most citrus and related plants tested are susceptible; only a few species developed certain types of persistent symptoms, and none, except satsuma, produced the leaf-cupping or downward leaf-bending typical of satsuma dwarf disease. In the present studies, seedlings of these species were repeatedly tested, and the reactions, including persistent symptoms, were not the same as those of satsuma dwarf disease on satsuma.

Kishi (2) found that an SDV component mechanically returned from herbaceous plants to citrus produced nonpersistent spotting and mottling on rough lemon seedlings and mottling accompanied by crinkling on trifoliolate orange. He therefore agreed with the previous description by Miyakawa (4), and concluded that the symptoms on rough lemon were induced by the virus component causing satsuma dwarf disease.

In the current study, rough lemon, grapefruit, and Dweet tangor showed

atypical nonpersistent symptoms following inoculation with certain virus isolates.

Some differences in symptoms were found on citrus plants inoculated with different isolates of SDV. SDV 1, 3, 4, and 5 produced symptoms of similar intensity on the test plants, but SDV 2 generally caused clearer and more severe symptoms. In contrast, SDV 6 caused very weak symptoms on rough lemon but none on grapefruit and Dweet tangor despite its severe effect on satsuma. It is accordingly suggested that there are strains of satsuma dwarf virus, although proof is lacking that the virus component responsible for symptoms on rough lemon and other citrus plants actually causes satsuma dwarf disease.

On satsuma seedlings or budlings, all isolates including NDV and CMV produced basically the same symptoms. Therefore, despite some observed differences in symptoms in herbaceous hosts, it is probable that all these isolates are related and can cause satsuma dwarf disease in satsuma.

From the results obtained to date, it is apparent that the virus associated with satsuma dwarf has a wide host range in citrus and causes symptoms in satsuma that are specific for that species. Also, based on the symptoms in satsuma, it is suggested that the SDV, NDV, and CMV virus isolates can all be called satsuma dwarf virus even though some were obtained from different host plants with atypical symptoms. The writer concludes that SDV isolates

1 to 5 (and possibly SDV 6) should be designated as the "A" or common strain of satsuma dwarf virus.

ACKNOWLEDGMENT.—The author

wishes to thank Prof. E. C. Calavan, University of California, for critical reading and correcting of the manuscript in English.

### *Literature Cited*

1. KISHI, K. 1967. Studies on the indicator plants for citrus viruses. III. Experiments on indexing for satsuma dwarf with sesame plant. IV. On the properties of the sap-transmissible virus associated with satsuma dwarf and some other virus-like diseases. Bull. Hort. Res. Station Series A (Hiratsuka) 6: 105-31.
  2. KISHI, K. 1968. Studies on the indicator plants for citrus viruses. V. Retransmission of the causal virus of satsuma dwarf from herbaceous host to citrus. Ann. Phytopathol. Soc. Japan 34: 224-30.
  3. KISHI, K., and TANAKA, S. 1964. Studies on the indicator plants for citrus viruses. II. Mechanical transmission of the virus, causing satsuma dwarf, to sesame (*Sesamum indicum* L.). Ann. Phytopathol. Soc. Japan 29: 142-48.
  4. MIYAKAWA, T. 1969. Susceptibility of Citrus spp. and the other related plants to the Satsuma dwarf virus. Ann. Phytopathol. Soc. Japan 35: 224-33.
  5. TANAKA, S. 1968. Satsuma dwarf. In J. F. L. Childs (Chmn.), Indexing procedures for 15 virus diseases of citrus trees. USDA Agr. Handbook No. 333: 56-59.
  6. TANAKA, S., and KISHI, K. 1963. Studies on the indicator plants for citrus viruses. I. Mechanical inoculation on leguminous plants with sap from satsuma dwarf tree. Ann. Phytopathol. Soc. Japan 28: 262-69.
  7. YAMADA, S., and SAWAMURA, K. 1952. Studies on the dwarf disease of satsuma orange, *Citrus unshiu* Marcov. (Preliminary report.) Hort. Div., Tokai-Kinki Agr. Expt. Sta. Bull. 1: 61-67.
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