Evidence for a Relationship among the Viruses of Satsuma Dwarf, Citrus Mosaic, Navel-Infectious-Mottling, Natsudaidai Dwarf, Citrus Variegation, and Citrus Crinkly Leaf

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THIS PAPER is concerned with studies in which evidence for a close relationship among the viruses of satsuma dwarf (SDV), citrus mosaic (CMV), navel-infectious-mottling (NIMV), and natsudaidai dwarf (NDV) was obtained. It also presents evidence for a somewhat less close relationship of citrus-crinkly-leaf virus (CCLV) and citrus-variegation virus (CVV) to SDV.

The main symptoms of citrus mosaic (3, 7)—green blotches or ring spots in the rind of fruit that are developing mature color—appear in late

September in fruit of early varieties and in October in fruit of late varieties of satsuma. The boat- or spoon-shaped leaves characteristic of satsuma dwarf appear only in spring flushes of trees infected with CMV.

Leaves of all flushes from trees infected with NIMV develop mottling, large, diffuse, chlorotic blotches, and often have brown spots on their undersurfaces. Sometimes yellow ring spots or blotches are also found (7). Many fruit drop after the June drop, and therefore severely affected trees yield poorly.

The main symptoms of natsudaidai dwarf are curling, crinkling, and variegation of young leaves (7). Like those of satsuma dwarf and citrus mosaic, they occur only in spring flushes of field trees.

Materials and Methods

INOCULA.—The sources of SDV, CMV, NIMV, and NDV were diseased trees at the Laboratory of Plant Pathology, Okitsu Branch, Horticultural Research Station originating, respectively, from Fujieda City in Shizuoka, Arita City in Wakayama, Katsuragi Town in Wakayama, and Hagi City in Yamaguchi Prefecture. Previous indexing in West Indian lime plants demonstrated that trees affected by any of these diseases usually carry tristeza virus (8). Indexing of field trees for the viruses we wished to study was often hampered by development of severe tristeza virus symptoms, confounding symptoms of the other viruses. In these cases, it was very difficult to detect specific symptoms of viruses other than that of tristeza in inoculated seedlings. To obviate this difficulty, buds from field-source trees carrying the other viruses were grafted to trifoliate orange seedlings, and then newly developed buds of the trifoliate orange seedlings were used as inocula. California strains of CCLV and CVV, imported from France, were compared with SDV and other viruses by making sap-inoculations to herbaceous plants.

INDICATOR PLANTS.—Two-year-old seedlings of West Indian lime, satsuma, natsudaidai, Eureka lemon, rough lemon, Etrog citron, Marsh seedless grapefruit, kabusu, Trovita sweet orange, and trifoliate orange were used as indicator plants. They were grown in earthen pots in an insect-proof greenhouse. Four replications were made for each trial. Five to 10 seedlings of white sesame, Satisfaction kidney bean, and Blackeye cowpea, respectively, were used for each sap-inoculation test.

BUD-INOCULATION TO CITRUS SEEDLINGS.—Citrus seedlings were graft-inoculated with buds of source plants in both sides of the main stem 3 cm above the ground, and were usually cut back to a single stem to force new shoot growth. Some inoculated plants were incubated in an air-conditioned greenhouse at about 23°C (6) and others in a non-air-conditioned greenhouse. Symptoms in the plants were observed 3–12 months after inoculation.

SAP-INOCULATION TO HERBACEOUS PLANTS.—Soft young leaves of source plants were cut into small pieces and ground in a mortar in the presence of the same volume of Sorensen's

phosphate buffer solution pH 7.0. Cotyledons of white sesame and newly developed primary leaves of kidney bean and cowpea were dusted with 500-mesh carborundum, rubbed with absorbent cotton containing prepared sap, and rinsed with tap water. These inoculated plants were incubated in an air-conditioned greenhouse at about 23°C (1). Symptoms were observed 7–10 days after inoculation.

Results and Discussion

The clear-cut reactions of various citrus and herbaceous plants inoculated with SDV and other viruses are shown in Tables 1 and 2.

WEST INDIAN LIME.—Almost all seedlings inoculated directly from field sources of SDV, CMV, NIMV, and NDV exhibited severe vein clearing in their new leaves and severe stunting of new growth. Some of them also developed vein corking, except for those inoculated with NDV. On the other hand, seedlings inoculated with buds of trifoliate orange subcultures showed scattered vein clearing but no other symptoms. The results suggest that the 4 diseased source trees carried mild and severe strains of tristeza virus simultaneously and that the severe strain was filtered out by the trifoliate orange seedlings.

EUREKA LEMON.—Seedlings inoculated with the field-source trees of SDV, CMV, and NIMV showed very severe yellowing and stunting of new growth, but those inoculated with NDV did not show such symptoms, indicating that the severe reaction in lime and lemon seedlings inoculated directly from field-source trees of SDV, CMV, and NIMV is caused by a seedling yellows component of tristeza virus. On the other hand, many circular clear spots —very similar to those already reported by Fraser in Australia for CCLV (2)—were found in newly developed

TABLE 1. SYMPTOMS IN VARIOUS CITRUS SEEDLINGS INOCULATED FROM TREES INFECTED WITH VIRUSES OF SATSUMA DWARF (SDV), CITRUS MOSAIC (CM), NAVEL-INFECTIOUS-MOTTLING (NIMV), AND NATSUDAIDAI DWARF (ND)

Seedling	Inoculum	SD	CM	NIMV	ND
West Indian lime	F[a]	Vc, Vco, St	Vc, Vco, St	Vc, Vco, St	Vc, St
	T	Vc	Vc		Vc
Eureka lemon	F	Y, St	Y, St	Y, St	
	T	Ccs		Ccs	Ccs
Satsuma	F, T	Bl, C	Bl, C	Bl, C	Bl, C
Rough lemon	F, T				C, V
Etrog citron	F				C, V
Marsh grapefruit	T		Ln, Y, V	M	
Natsudaidai	F, T	Crs	Ln, Y, V	Ln', C, V	
Kabusu	F		Ln, Y		
Trovita orange	F, T		Ln, Y, V	M	
Trifoliate orange	F		V		V

a. F, field-source trees; T, trifoliate subculture from field-source trees; C, crinkle; Bl, boat-shaped leaf; Ccs, circular clear spots; Crs, chlorotic ring spots; M, mottling; Ln, leaf narrowing; Ln', leaf narrowing with irregular leaf edges; St, stunting; V, variegation; Vc, vein clearing; Vco, vein corking; Y, yellowing.

TABLE 2. SYMPTOMS IN SOME HERBACEOUS PLANTS INOCULATED WITH VIRUSES OF SATSUMA DWARF (SDV), CITRUS MOSAIC (CMV), NAVEL-INFECTIOUS-MOTTLING (NIMV), NATSUDAIDAI DWARF (NDV), CITRUS-CRINKLY-LEAF (CCLV), OR CITRUS VARIEGATION (CVV)

Seedling	Symptoms	SDV	CMV	NIMV	NDV	CCLV	CVV
Sesame							
Inoculated leaf	Necrotic lesions	+	+	+	+	−	−
Noninoculated	Vein clearing	+	+	+	+	−	−
upper leaf	Vein necrosis	+	+	+	+	−	−
	Bending of midrib	+	+	+	+	−	−
	Malformation	+	+	+	+	−	−
Blackeye cowpea							
Inoculated leaf	Chlorotic spots	+	+	+	+	−	+
Noninoculated	Mottling	+	+	+	−	−	+
upper leaf	Curling	+	+	+	−	−	+
	Diffuse yellow vein banding	+	−	−	−	−	−
	Fine vein yellowing	−	−	−	−	+	−
Stem and petiole	Necrotic streak	+	+	+	−	+	+
Satisfaction kidney bean							
Noninoculated	Vein clearing	+	+	+	−	+	−
upper leaf	Vein necrosis	+	+	+	−	+	−
	Malformation	+	+	+	−	+	−
	Yellow vein banding	−	−	−	−	−	+
	Crinkle	−	−	−	−	−	+
	Variegation	−	−	−	−	−	+

leaves of Eureka lemon seedlings inoculated with trifoliate orange subcultures of these viruses.

SATSUMA.—All seedlings inoculated from field-source trees or trifoliate orange subcultures showed typical boat-shaped leaves and slight crinkling of new leaves. These symptoms were more severe in the satsuma seedlings inoculated with NDV than in those inoculated with SDV, CMV, or NIMV.

OTHER CITRUS SPECIES.—The following results were obtained by graft-inoculations from field-source trees and trifoliate orange subcultures into various kinds of citrus seedlings. 1. CMV caused characteristic leaf narrowing, accompanied by marked yellowing of new growth of Marsh grapefruit, natsudaidai, kabusu, and Trovita orange, and variegation in new leaves of Marsh grapefruit, natsudaidai, Trovita orange, and trifoliate orange. 2. NIMV caused characteristic leaf narrowing with irregular leaf margins, crinkle, and variegation in new leaves of natsudaidai, and mottling accompanied by many small brown spots on the undersurface of leaves of Marsh grapefruit and Trovita orange seedlings. This type of mottling is identical with that in leaves of navel orange trees infected with NIMV. 3. NDV also caused very clear-cut variegation and crinkling in new leaves of rough lemon seedlings and Etrog citron budlings.

All these symptoms remained in new leaves after maturity.

SESAME.—White sesame is known to be the best indicator plant for SDV (4). Sap-inoculation tests with sesame plants were therefore made in order to obtain additional evidence regarding the presumed relationship of CMV, NIMV, and NDV to SDV. Seedlings inoculated with these 3 viruses exhibited necrotic local lesions in treated leaves, and vein clearing, vein necrosis, bending of the midrib, and malformation of the upper systemically infected leaves. These symptoms are identical with those caused by SDV. In contrast, the California strains of CCLV and CVV did not cause such symptoms in leaves of sesame.

LEGUMINOUS PLANTS.—In addition to sesame, leguminous plants such as Blackeye cowpea and Satisfaction kidney bean are known to be suitable test plants for SDV (5). As shown in Table 2, Blackeye cowpea seedlings developed comparatively large chlorotic spots in inoculated leaves; mottling, curling, and diffuse yellow vein banding in upper leaves; and necrotic streaks in stems and petioles when inoculated with SDV, CMV, or NIMV. Seedlings of Satisfaction kidney bean inoculated with these viruses exhibited vein clearing, vein necrosis, and malformation of upper leaves. However, NDV caused only slightly chlorotic spots in a few inoculated leaves of blackeye cowpea seedlings but no symptoms in seedlings of kidney bean. On the other hand, CCLV caused chlorotic spots in sap-inoculated cowpea leaves and yellowing of the very fine veins of upper leaves; CVV caused mottling and curling of upper leaves. Satisfaction kidney bean exhibited vein clearing, vein necrosis, and malformation when inoculated with CCLV; it developed crinkle, variegation, and yellow vein banding when infected with CVV.

Conclusions

From the occurrence of many circular clear spots on newly developed leaves of Eureka lemon and the similarity of symptoms produced on leguminous plants, it is thought that SDV may be a strain of CCLV. If so, it is somewhat different from the California strain because the latter caused no reaction in white sesame seedlings.

In contrast, CMV, NIMV, and NDV caused clear variegation in leaves of some citrus varieties such as rough lemon and natsudaidai. This indicates that these viruses may be strains of CVV somewhat different from the California strain as indicated by their different effect in Satisfaction kidney bean.

Seedlings of satsuma and other Japanese citrus, which are used as indicator plants in our laboratory, are now being used in comparative studies with the California strain of both CCLV and CVV.

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