Protective Interference of Mild Strains of Citrus Tristeza Virus Against a Severe Strain in Morita Navel Orange[z]

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ABSTRACT. Nursery plants of citrus tristeza virus (CTV)-free Morita navel orange obtained by thermotherapy were individually preinoculated with mild strains of CTV, collected from citrus trees in orchards, or artificially produced by heat treatment of Morita navel orange infected with a severe strain of seedling yellows CTV. They were challenge-inoculated with a severe strain of seedling yellows CTV by the vector (*Toxoptera citricidus*) or by side-graft inoculation. In the first experiment, the occurrence of stem pitting on four plants preinoculated with M-10 (HM-55 from Hassaku, Hiroshima Pref.) mild strain was slight 4 yr after challenge-inoculation, and two of four plants showed mild symptoms on indexing to Mexican lime seedlings. Fruit size and production of CTV-free plants and plants preinoculated with mild strain were larger than those of plants with the severe strain, but there was little difference in trunk girth of plants between treatments. In the second experiment, the M-23(A) mild strain produced by heat treatment was effective 2 to 3 yr after challenge-inoculation, based on mild stem pitting observed and mild symptoms on Mexican lime seedlings used to index challenged plants. The M-10 and M-23(A) mild strains appear promising to protect Morita navel orange trees from severe strain challenge under the field conditions.
Index words. Artificial production of mild strains of CTV, *Toxoptera citricidus*, stem pitting.

Conversion from Satsuma mandarin to other varieties such as Hassaku, navel orange, Miyauchi-Iyo, Natsudaidai or other cultivars has been carried out in Japan because of overproduction of Satsuma mandarin, and low prices since 1972. Unfortunately, these varieties are susceptible to citrus tristeza virus (CTV), and tree vigor, fruit production, and fruit size are badly affected. Converted navel oranges were infected by severe strains of seedling yellows CTV and severe stem pitting generally occurred. Stem pitting on twigs of navel orange was inversely related to tree vigor, fruit production and fruit size (14). Even if CTV-free nursery plants are replanted in the fields, they will be quickly infected by severe strains of CTV because the vectors, *Toxoptera citricidus* Kirk. and *Aphis gossypi* Glov. (8), are common in Japanese citrus orchards.

The recognition of mild strains of CTV and cross-protection with mild strains of CTV against severe strains were reported by Grant (5). Since then, many studies on utilization of mild strain to avoid damage caused with CTV has been caried out worldwide (1, 3, 4, 7, 9, 10, 11, 15, 16, 17).

In Japan, the damage to Hassaku was first reported and then associated with severe CTV. To avoid CTV damage to Hassaku, a vigorous and fruitful tree was selected from a farmer's orchard in Hiroshima prefecture. It was shown that this tree was infected by a mild strain of CTV (HM-55) (12, 13). This tree became the mother tree for Hassaku, and many nursery plants were multiplied and distributed to farmers. Most of these are vigorous and productive at present.

Navel orange usually lacks vigor, produces small-sized fruit, and requires a devoted farmer to cultivate in Japan. To resolve these problems, a plant of Morita navel orange, the main strain of navel orange in Japan, was freed of CTV and CTV-free plants were preinoculated by a mild strain. Preinoculated nursery plants were challenge-inoculated with severe strains by side-grafting with small twigs or inoculation with *T. citricidus*. Periodic indexing on Mexican lime, surveys for stem pitting, tree vigor, and fruit size have been carried out, and in this paper we describe our preliminary results.

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MATERIALS AND METHODS

Test plants. A Morita navel orange infected with a severe strain of seedling yellows CTV was heat-treated at 45/35C to inactivate CTV (6). CTV-free nursery plants were preinoculated with each mild strain. Infection was verified after 3 months by enzyme-linked immunosorbent assay (ELISA) (2). Scions cut from these plants were grafted on trifoliate orange rootstock.

Mild strains of CTV. Four mild strains were used (table 1). M-8, M-10, and M-16 mild strains were obtained from citrus trees in orchards (18, 19). M-23(A) was obtained by a 14-day heat treatment at 45C day/35C night of Morita navel orange infected by a severe strain of seedling yellows CTV.

Cross-protection trials

First experiment. Nursery plants of Morita navel orange preinoculated with M-8, M-10 and M-16 were challenge-inoculated by severe seedling yellows CTV by two methods: 1) side-grafting with a small infected twig which was removed after 30 days (May 1981), and 2) feeding by *T. citricidus* (total of 20 or 40 aphids per plant) for 2 days in May 1981 and 1982. In October 1983, each plant was transplanted into a plastic container (40 x 57 x 30 cm) and put outside. Each plant was indexed on Mexican lime seedlings every year, and also examined for stem pitting. Weight, sugar content (Brix), and acid content of fruits were determined. Four plants per treatment were tested.

Second experiment. CTV-free nursery plants of Morita navel orange preinoculated by M-23(A) of mild strain were challenge-inoculated by severe strains with *T. citricidus* (total of 5, 10, 20 aphids) in May and July 1982 and April 1983. In September 1984, each plant was transplanted into a plastic container. Indexing on Mexican lime seedlings, examination for stem pitting and fruit analyses were carried out as in the first experiment.

RESULTS

Production of mild strain of CTV by heat-treatment. Small twigs of Morita navel orange heat-treated at 45/35C for 14 to 35 days were side-grafted on Mexican lime seedlings. Only one Mexican lime seedling which was side-grafted with heat-treated tissue for 14 days showed mild symptoms (table 2). CTV was detected by ELISA in this plant. The isolate was transmitted by *T. citricidus*, and was designated M-23(A).

Cross-protection trials. In November 1985, 4 yr after challenge-inoculation, the first experiment was read. Stem pitting ratings on twigs of plants preinoculated with the mild M-8 or M-16 strains and followed by challenge-inoculation, and CTV-free plants challenge-inoculated with severe strain were 50 to 59 and 51 to 72, respectively (table 3). But ratings of stem pitting in plants with M-16 only and nonchallenged CTV-free plants were 6 and 0, respectively. On the other hand, stem pitting ratings in plants with M-8 and M-10 without challenge-inoculation were 18 and 27,

TABLE 1
MILD CITRUS TRISTEZA VIRUS (CTV) STRAINS USED IN CROSS-PROTECTION TESTS

No.	CTV strain	Source	Remarks
M-8	CTV-SP	Vietnam Pummelo	Seedling
M-10	CTV-SP	Hassaku	Dr. Sasaki
M-16	CTV-SY	Okitsu No. 17	Hybrid seedling
M-23(A)	CTV-SY	Morita navel orange	Produced by heat treatment; separated by aphid

TABLE 2
PRODUCTION OF MILD STRAINS OF CITRUS TRISTEZA VIRUS (CTV) BY HEAT TREATMENT OF SEVERE STRAINS

Period for heat treatment (days)[z]	No. of plants grafted	No. of CTV-free plants[y]	No. of CTV-infected plants	CTV symptoms[x]
14	20	19	1	mild
21	20	19	1	severe
28	20	20	0	—
35	19	19	0	—

[z]Heat-treatment at 45/35 C (day/night).
[y]Detected by enzyme-linked immunosorbent assay.
[x]Judged by symptoms on Mexican lime seedlings.

and rating in challenged plants with M-10 was 23. There was no difference in occurrence of stem pitting between challenge inoculations with *T. citricidus* and side-grafting of tissue.

The results of indexing with Mexican lime seedlings in 1982, 1983 and 1984 showed that most plants infected with M-16 and challenge-inoculated, and challenge-inoculated CTV-free plants contained severe CTV. Limes inoculated with M-8 only, and challenged plants with M-8 showed severe symptoms, but occurrence of stem pitting on plants with M-8 only was less (rating: 18) than it was in challenged plants (rating: 59). Plants with M-16 only showed mild symptoms. On the other hand, plants with M-10 only, and challenged plants with M-10 showed severe or mild symptoms.

There were no distinct differences in trunk girth of plants, sugar and acid contents of fruit among treat-

TABLE 3
CROSS-PROTECTION TESTS BETWEEN MILD AND SEVERE STRAINS OF CITRUS TRISTEZA VIRUS (CTV) IN MORITA NAVEL ORANGE (FIRST EXPERIMENT)

CTV		Indexing[x]	Stem pitting[w]			Trunk girth (cm)	Fruit per tree (Dec 1985)		Fruit per tree (Dec 1986)	
Preinoculated[z]	Challenged[y]		Nov 83	Nov 84	Nov 85	Aug 86	No.	Avg. wt. (g)	No.	Avg. wt. (g)
M-16	S-5A2	S,S,M,S	37	54	50	9.2	5.3	226	8.0	186
	S-5A3	S,x,S,M	26	14	61	9.7	4.3	278	11.0	153
	S-5G	S,S,S,S	40	25	53	9.1	5.8	218	8.0	201
	—	M,M,M,M	1	0	6	9.2	8.8	250	11.8	279
M-8	S-5A2	S,S,S,S	7	15	59	9.4	3.5	266	12.8	178
	—	S,S,S,S	1	4	18	10.0	7.8	248	10.3	296
M-10	S-5A2	M,M,S,S	12	15	23	9.2	7.5	216	11.0	224
	—	M,M,S,M	12	7	27	9.1	6.3	251	16.3	217
Free	S-5A2	S,S,S	13	27	51	9.3	4.3	244	12.3	153
	S-5A3	S,S,S,S	55	34	72	8.9	4.5	216	11.0	179
	S-5G	S,S,S,S	26	43	65	8.9	3.3	260	15.3	167
	—	F,F,F,F,F	0	0	0	9.2	5.4	288	11.3	284

[z]Scions preinoculated with mild strains of CTV were grafted on trifoliate orange seedlings, May 1980.
[y]Preinoculated nursery plants were challenge-inoculated by severe strains with *Toxoptera citricidus* (A2: total 20 aphids, A3: total 40 aphids per plant) in May 1981 and 1982 or grafting for 30 days in May 1981 (G).
[x]Result of individual plant indexing on Mexican lime seedlings in October 1982, 1983, and 1984. M = mild symptom, S = severe symptom, F = no symptom, x = died.
[w]Figures represent degree of stem pitting observed on 3 to 10 twigs per plant. 0 = no stem pitting, 100 = very severe stem pitting.

ments, but fruit size and production per tree of CTV-free plants and plants infected with mild strains were larger than in plants infected with the severe strains.

In September 1985, the second experiment was read 2 to 3 yr after challenge-inoculation. The stem pitting rating in twigs of plants preinoculated with M-23(A) only, and M-23(A) and challenge-inoculated with 5, 10, 20 aphids per plant were 0 and 0 to 7, respectively. By indexing with Mexican lime seedlings in 1983, 1984 and 1985, severe symptoms were detected from three challenged plants, but very mild symptoms were detected from the others and plants with M-23(A) only.

DISCUSSION

The interim results 2 to 4 yr after challenge-inoculation showed that severe stem pitting occurred in CTV-free plants inoculated with a severe strain of seedling yellows CTV, and fruit size and production of plants infected with the severe strain were reduced compared to plants infected with the mild strain alone and CTV-free plants. These confirm the survey results that severity of stem pitting in navel orange in the farmer's orchards was correlated with small fruit size and low fruit production. But occurrence of stem pitting is slight on twigs of plants preinoculated with M-10 or M-23(A) mild strains of CTV and challenge-inoculated, and indexing on Mexican lime seedling indicated that they carry mild isolates.

There are several reports that cross-protection was affected by differences in the number of the vectors and the severity of the strain of CTV used for challenge-inoculation. Sasaki (13) reported that cross-protection was not distinct when more than 15 *T. citricidus* per plant were used for challenge-inoculation. Therefore, if few *T. citricidus* were fed at a time for challenge-inoculation instead of the more severe side-grafting or feeding of many *T. citricidus* used in our experiments, it is presumed that cross protection would be more effective. Therefore, it should be very important to keep the vector population low when plants preinoculated with mild strains are planted in the field.

From these results, the M-10 and M-23(A) mild strains of CTV are promising for protective interference against severe strains of CTV for a long term in navel orange. Practical trials of navel orange cultivation with nursery plants preinoculated with these mild strains under the field condition are being carried out.

TABLE 4
CROSS-PROTECTION TESTS BETWEEN MILD AND SEVERE STRAINS OF CITRUS TRISTEZA VIRUS (CTV) IN MORITA NAVEL ORANGE (SECOND EXPERIMENT)

CTV		Indexing[x]	Stem pitting[w] (Dec 1985)	Trunk girth (cm) (Aug 1986)	Fruit per tree (Dec 1986)	
Preinoculated[z]	Challenged[y]				No.	Avg. wt. (g)
M-23(A)	S-5A1 [a)]	M,M,S	3.3	5.8	7.5	230
	S-5A2 [a)]	M,M,M	0	6.4	5.0	295
	S-5A3 [a)]	M,S	5.0	6.3	8.5	241
	S-5A3 [b)]	M,M,M,S	7.0	5.3	10.0	223
	—	M,M	0	6.7	13.5	236

[z]Scions preinoculated with mild strain of CTV were grafted on trifoliate orange seedlings, May 1980.
[y]Preinoculated nursery plants were challenge-inoculated by severe strains with *Toxoptera citricidus* (A1: 5 aphids, A2: 10 aphids, A3: 20 aphids). [a)] Challenge-inoculated in April, 1983. [b)] Plants preinoculated with M-23, and challenge-inoculated in May and July, 1982.
[x]Result of individual plant indexing on Mexican lime seedlings in October 1983, August 1984 and October 1985. M = mild symptom, S = severe symptom.
[w]Figures represent degree of stem pitting observed on 3 to 10 twigs per plant. 0 = no stem pitting, 100 = very severe stem pitting.

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