The Association of Tatterleaf Virus with Budunion Crease of Trees on Trifoliate Orange Rootstock

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ABSTRACT. A budunion crease of citrus trees grafted on trifoliate orange has been associated with inoculum sources carrying tatterleaf (citrange stunt) virus. Inoculation tests have been made since 1976 under greenhouse conditions using sweet orange and satsuma mandarin trees on trifoliate rootstock as indicators. Of 30 isolates of tatterleaf virus tested, three have not shown any visible symptoms of budunion crease on these indicator plants. All agents causing the budunion crease, tatterleaf and citrange stunt reactions were simultaneously transmitted from infected herbaceous plants to citrus by mechanical inoculation.

Index words. Citrus excelsa, citrange stunt, mechancial transmission.

Tatterleaf virus, first described by Wallace and Drake (12, 13) in California, was later found in Florida (4), Japan (7), Australia (3) and South Africa (2). Sources of this virus carry components causing variable type of symptoms, *i.e.*, tatterleaf in *Citrus excelsa*, citrange stunt in citranges (5, 12), and budunion crease of trees on trifoliate orange (1, 7).

Most trees of Ponkan and Tankan mandarins grown in Japan are infected by tatterleaf virus (8). This virus probably originated in China (southern mainland and Taiwan), and was introduced to Japan in infected trees and budwood during the past 90 yr (11).

Recent topworking of satsuma trees to new citrus cultivars has resulted in an increased incidence of budunion crease, with associated symptoms of a tree decline. The symptoms are highly injurious to commercial plantings.

Since the 1970's, inoculum sources that induce both tatterleaf and citrange stunt symptoms have been collected. These were assumed to carry components causing budunion crease of trifoliate orange-rooted citrus trees (1, 5, 7).

This paper reports greenhouse experiments to test the relationship between tatterleaf and budunion crease.

MATERIALS AND METHODS

Inoculum sources. Isolates of tatterleaf (citrange stunt) virus negative for budunion crease were designated as BUC-negative No. 1, 2 and 3, respectively. These isolates were obtained from different areas of Japan. The BUC-negative No. 1 is from a citrus hybrid (originally bred and released by the National Horticultural Research Institute as breeding No. 6781) planted by a grower in Tokushima. Isolate No. 2 was obtained from a Ponkan tree planted in Kochi, and isolate No. 3 was from a tree of an unidentified citrus cultivar grown in the field at the Kuchinotsu Station (a branch station of the National Horticultural Research Institute).

Okitsu Wase satsuma [an isolate used previously (8)] was used as a standard isolate of tatterleaf which causes budunion crease (BUC-positive). Supplementary isolates (Morita and F2428 Ponkan trees) were also used as the budunion crease-positive controls.

Inoculation tests to citrus trees grafted on trifoliate orange. One-yr-old greenhouse-grown satsuma mandarin and sweet orange trees on trifoliate orange rootstock were used. Inoculations were made by inserting inoculum buds into T-slits in the bark of the plants. Inoculated plants were kept in the greenhouse for at least 2 yr for symptom observation and bark of the plant was removed at the budunion every year after inoculation to detect budunion crease.

Indexing for tatterleaf virus in noncreased plants. To confirm existence of tatterleaf (citrange stunt) virus infectivity in plants inoculated by BUC-negative isolates, healthy buds of Rusk citrange and *C. excelsa* were top-grafted onto the plants. After top-grafting, the plants were kept at 20 to 24 C to induce leaf symptoms in the new shoots of the indicators.

Mechanical inoculation of cowpea, bean and citrus plants. New leaves collected from previously inoculated citrus plants were used for sap inoculation on the primary leaves of Blackeye cowpea and Masterpiece bean plants. Inoculated plants were kept at 20 to 22 C for 2 weeks. Local lesions were compared for the tatterleaf virus isolates used in this tests.

Bean leaves mechanically inoculated with the Okitsu Wase isolate and showing necrosis on advanced young leaves were used as the inoculum source to back inoculate rough lemon seedlings and Valencia sweet orange on trifoliate root. These were razor (knife)-cut inoculated with the infected bean extract.

RESULTS AND DISCUSSION

Thirty inoculum sources producing symptoms in *Citrus excelsa* and Rusk citrange plants were also tested for budunion crease by inoculating to sweet orange and satsuma mandarin on trifoliate orange rootstock. The inoculum source trees were from different areas of Japan, and some were already showing budunion crease in trees on trifoliate orange rootstock. Of 30 isolates tested, three sources have not produced budunion crease. These inoculum sources were used in subsequent tests as the budunion crease-negative (BUC-negative) isolates.

Experiments No. 1 and 2 originally were done to index for the tatterleaf virus and the budunion crease agent. When some sources failed to cause budunion crease in trifoliate orange-rooted citrus trees, experiments 3, 4 and 5 were set up to confirm existence of budunion crease-negative isolates.

All of the BUC-positive isolates used in these tests produced budunion crease within 2 yr after inoculation (table 1). However in repeated inoculations made since 1976, BUC-negative isolates No. 1, 2 and 3 have produced no symptoms of budunion crease, even 3 to 4 yr after inoculation (fig. 1). Since a clear budunion crease was detected within 1 yr in young budded trees of Valencia sweet orange and satsuma mandarin, inoculated with BUC-positive isolates, these scion and rootstock combinations were considered good indicators.

After inoculation with BUC-negative isolates, inoculated, budded trees without budunion crease were repeatedly indexed for tatterleaf virus infection. When healthy buds of *C. excelsa* and Rusk citrange were top-grafted on these trees, typical symptoms of tatterleaf and citrange stunt were observed after several weeks. These symptoms were similar to those induced by BUC-positive isolates under favorable temperature conditions. In a previous paper (8), *C. excelsa* and Rusk citrange were recommended as the most reliable indicators for detecting the budunion crease agent, but these experiments raise a question about the correlation for all isolates.

A differential response in herbaceous indicator plants between BUC-positive and negative isolates was observed. Somewhat different sizes of local lesions in bean plants were observed with the isolates tested. Local lesions induced by the Okitsu Wase isolate were generally larger than others.

Bean leaves infected by the Okitsu Wase isolate (BUC-positive) were used as inoculum for razor (knife)-cut inoculation to rough lemon seedlings and Valencia sweet orange trees budded on trifoliate orange root. One of three of each became infected and showed tatterleaf and citrange stunt symptoms in subinoculated *C. excelsa* and Rusk citrange. The infected rough lemon seedlings was found

TABLE 1
VARIABILITY OF BUDUNION CREASE (BUC) EXPRESSION IN TATTERLEAF (CITRANGE STUNT) VIRUS ISOLATES

Experiment No.	Virus isolate	Scion[z]	Inoculation date	Observation date	No. plants inoculated	Budunion crease[y] None visible Negative	Budunion crease[y] Clearly Positive
1	BUC[w]-neg. No. 1	Satsuma	7/76	7/78	4	4	0
	BUC-neg. No. 3	Satsuma	7/76	7/78	5	5[x]	0
	Morita (BUC-pos.)	Valencia	7/76	7/78	5	0	5
2	BUC-neg. No. 2	Valencia	6/77	7/79	3	3	0
	BUC-neg. No. 3	Valencia	9/77	6/81	3	3	0
3	BUC-neg. No. 1	Valencia	6/81	6/84	4	4	0
	BUC-neg. No. 2	Valencia	6/81	6/84	4	4	0
	BUC-neg. No. 3	Valencia	6/81	6/84	3	3	0
	F2428 (BUC-pos.)	Valencia	6/81	6/84	3	0	3
	Okitsu Wase (BUC-pos.)	Valencia	6/81	6/84	3	0	3
4	BUC-neg. No. 1	Valencia	5/83	7/85	3	3	0
	BUC-neg. No. 2	Valencia	5/83	7/85	3	3	0
	BUC-neg. No. 3	Valencia	5/83	7/85	3	3	0
	Okitsu Wase (BUC-pos.)	Valencia	5/83	7/85	3	0	3
5	BUC-neg. No. 1	Valencia	3/84	7/86	4	4	0
	BUC-neg. No. 2	Valencia	3/84	7/86	4	4	0
	BUC-neg. No. 3	Valencia	3/84	7/86	4	4	0
	Okitsu Wase (BUC-pos.)	Valencia	3/84	7/86	4	0	4
	Non-inoculated	Valencia	3/84	7/86	4	4	0

[z]All budded on trifoliate orange rootstock.
[y]Examined at the budunion after peeling the bark.
[x]Very slight or suspect.
[w]Budunion crease negative in previous tests.

positive for budunion crease by inoculation to Valencia sweet orange on trifoliate orange. Mechanically inoculated Valencia sweet orange on trifoliate orange showed a clear budunion crease 2 yr after inoculation.

Garnsey (6) reported that a virus component inducing tatterleaf symptoms in *C. excelsa* was mechanically transmitted from herbaceous hosts to citrus. In our tests, the virus components causing tatterleaf citrange stunt and budunion crease were simultaneously transmitted by razor-cut inoculation from infected bean to citrus plants. According to Usugi (personal communication) a partially purified inoculum of tatterleaf virus was knife-cut inoculated to citrus and produced all reactions including budunion crease. Roistacher (9) carried out extensive inoculation tests in an attempt to separate tatterleaf and citrange stunt complex, and the components were still not completely separated. However, Roistacher (10) has recently concluded that tatterleaf and citrange stunt are components of the same virus complex and suggests the original name "Tatter leaf" should cover all components of this virus complex. These results may support a hypothesis that the symptoms in different indicator plants are induced by a virus which has variable effects on citrus under some conditions. These studies also indicate that there is an

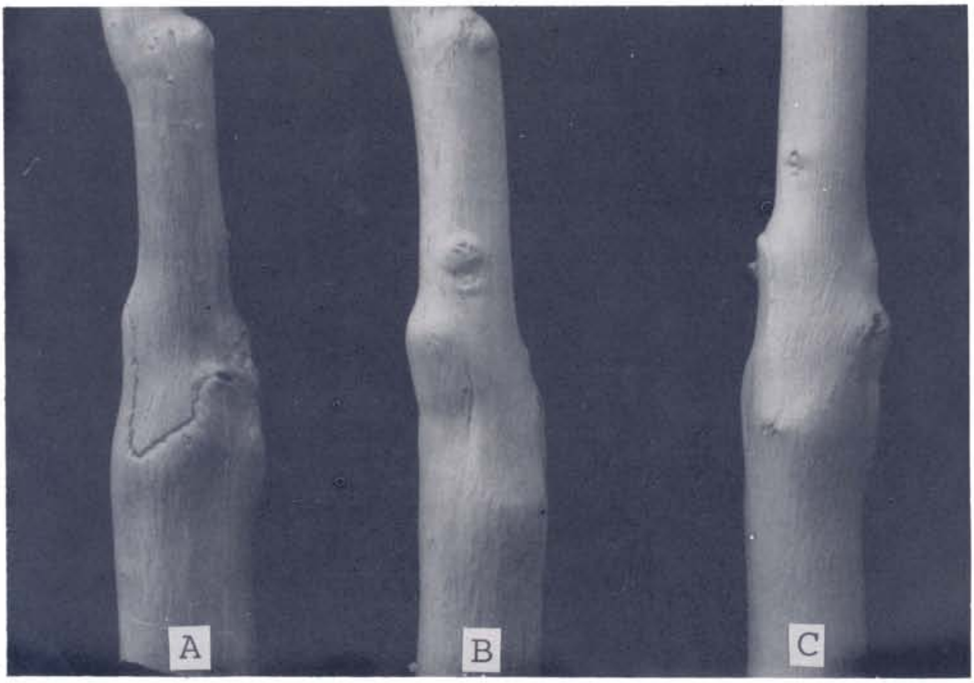

Fig. 1. Budunions of Valencia sweet orange on trifoliate orange rootstocks: A) a clear crease in plants infected with a budunion crease (BUC)-positive isolate from Okitsu Wase; B) normal union in plant infected with the BUC-negative isolate No. 2; and C) union of healthy, uninoculated control. All unions examined 24 months after inoculation.

additional component of the tatterleaf complex, since some isolates fail to produce the budunion crease symptom.

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