

# A Bud-Union Abnormality of Satsuma Mandarin on *Poncirus Trifoliata* Rootstock in Japan

T. Miyakawa and C. Matsui

In Japan the most widely used rootstock for citrus is *Poncirus trifoliata*. It has long been considered to be the best rootstock for Satsuma and many other citrus varieties. The unions of most Satsuma varieties on *P. trifoliata* are normal and the overgrowth of *P. trifoliata* rootstock has been known as a normal characteristic.

Recently a bud-union abnormality characterized by basal scion swelling (called "Dai-Make") and bud-union crease was noticed in a few lines of Satsuma on

*P. trifoliata*. One of the nucellar lines, Okitsu-Wase Satsuma, has been propagated since 1949. In 1962, progeny of the Okitsu-Wase were found with "Dai-Make" in Shizuoka Prefecture (Tachikawa *et al.*, 1968). In Nagasaki, an old-line Hayashi Satsuma also was found with similar symptoms of Satsuma scion swelling at the bud union. This paper reports preliminary studies to determine whether the factor inducing this bud-union disorder is transmissible.

## TRANSMISSION EXPERIMENTS WITH THE BUD-UNION DISORDER OF SATSUMA ON *P. TRIFOLIATA*

**Materials and Methods.** Three varieties of bud-union-creased Satsuma were collected from Nagasaki and Shizuoka, Japan and used as inoculum sources for transmission experiments.

Budlings of virus-free Satsuma, Valencia orange, Yuzu, and mild tristeza-infected *Citrus hassaku* on *P. trifoliata* were used as indicator plants. The budlings were grown in 13-cm-diameter clay pots in an insect-free greenhouse and later transplanted into larger pots if necessary. Greenhouse temperatures usually ranged from 20 to 30°C, but dropped to 18°C on the coldest winter nights. Maximum temperature occasionally reached 35°C on the hottest summer days.

Inoculations were made by inserting inoculum buds into T-slits in the bark of indicator plants.

**Results.** The results of transmission tests started from 1967 to 1974, using

three isolates from bud-union-creased Satsuma trees, are summarized in table 1. All three isolates (from Hayashi, Okitsu-Wase, and Noda Satsumas) were found to be graft-transmissible. Budlings examined by removing the bark across the bud union one year after inoculation showed a sharp deep ring encircling the trunk between the scion and rootstock of infected trees (fig. 1). The inoculated Satsuma budlings developed a swelling of the scion near the bud union within 3 to 4 years after inoculation (fig. 2).

Inocula from bud-union-creased Satsuma trees were also grafted into Valencia orange, Hassaku, and Yuzu on *P. trifoliata* rootstock. These scion-rootstock combinations also developed the bud-union crease following inoculation (table 2). It is apparent that the pathogen caused bud-union crease in several scion-rootstock combinations.

## IMMUNITY OF *P. TRIFOLIATA* TO THE PATHOGEN

Buds from *P. trifoliata* seedlings previously inoculated with infected Satsuma buds, in which the inocula still remained,

were grafted into healthy Valencia orange on *P. trifoliata* rootstock. No transmission of the pathogen were detected (table

TABLE 1  
RESULTS OF INOCULATION OF SATSUMA ON *P. TRIFOLIATA* ROOTSTOCK  
BY BUDS TAKEN FROM BUD-UNION-CREASED TREES

Experiment Number*	Inoculum Source	Plants Inoculated	Plants Infected†
1	Hayashi Satsuma, abnormal scions	3	3‡
	Hayashi Satsuma, healthy scions	3	0
	Checks	3	0
2	Hayashi Satsuma, abnormal scions	4	4
	Hayashi Satsuma, abnormal buds of rootstock§	4	0
	Hayashi Satsuma, healthy scions	4	0
3	Hayashi Satsuma, abnormal scions	2	2
	Okitsu-Wase Satsuma, abnormal scions	4	4
	Checks	4	0
4	Hayashi Satsuma, abnormal scions	2	2
	Hayashi Satsuma abnormal buds of rootstock§	3	0
	Noda Satsuma abnormal scions	3	2
	Checks	3	0

\*Experiment 1. Inoculated October, 1967; examined in 1973.

2. Inoculated July, 1972; examined in 1974.

3. Inoculated March, 1973; examined in 1975.

4. Inoculated February, 1974; examined in 1975.

†Definite crease developed at the bud union.

‡Definite scion swelling and crease detected.

§Buds from the shoots of *P. trifoliata* rootstock.

3). In other experiments, buds from shoots of the *P. trifoliata* rootstock of a tree with bud-union crease were inoculated into Satsuma on *P. trifoliata*. No transmission of the pathogen occurred in these cases either (table 1, Exp. nos. 2, 4).

However, when infected and virus-free Satsuma buds were simultaneously grafted into a *P. trifoliata* seedling, or when virus-free Satsuma scions were top-worked on shoots of *P. trifoliata* of the bud-union-creased trees, bud-union crease resulted.

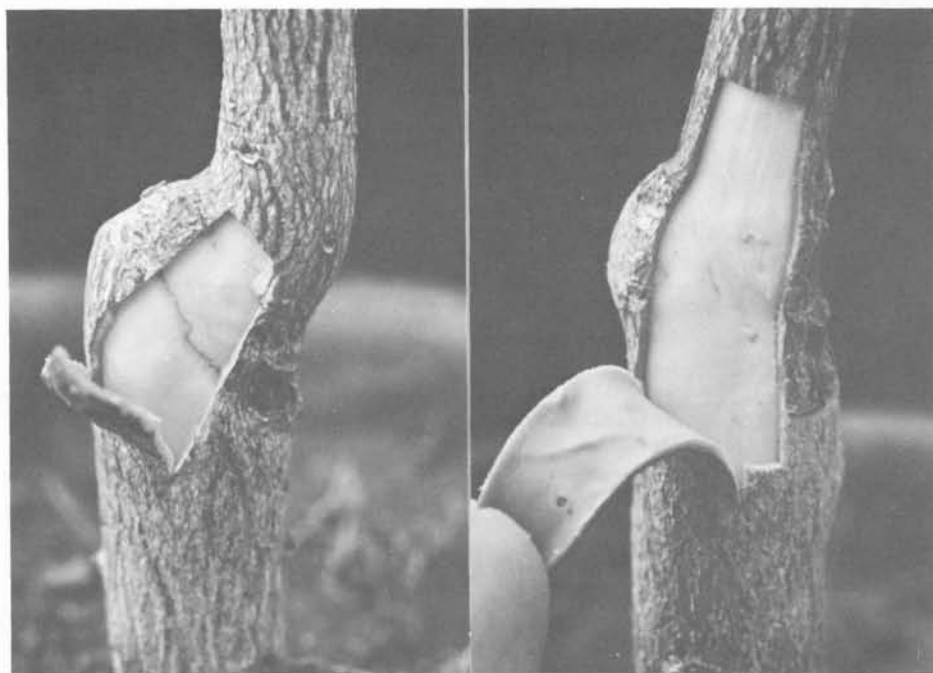


Fig. 1. Bud-union crease of Satsuma on *P. trifoliata* one year after inoculation (left); normal union (right).

#### INDEXING OF SUSPECTED VIRUSES ON *CITRUS EXCELSA*, TROYER CITRANGE, AND CITREMON

Seedlings of *C. excelsa* and citremon and budlings of Troyer citrange on Yuzu were inoculated with buds from the three inoculum sources. About 6 to 7 months after inoculation clear blotching and distortion of the leaves of Troyer citrange and citremon occurred in plants inoculated from Okitsu-Wase (fig. 3). Plants inoculated from Noda Satsuma exhibited slight symptoms. The symptoms in plants inoculated from Okitsu-Wase resembled those caused by citrange stunt virus (Wallace and Drake, 1963). Conversely, no symptoms were detected in plants inoculated from Hayashi Satsuma. Reaction of *C. excelsa* to inoculations from any of the sources were not clear due to severe tristeza reactions.

#### TRANSMISSION TO COWPEA, *VIGNA SINENSIS*

Young leaves collected from bud-union-creased Satsuma budlings were used for mechanical inoculations to cow-



Fig. 2. Scion swelling of Satsuma on *P. trifoliata* root, six years after inoculation.

TABLE 2  
REACTION OF SWEET ORANGE, HASSAKU, AND YUZU ON *P. TRIFOLIATA* TO THE PATHOGEN ASSOCIATED WITH THE BUD-UNION DISORDER OF SATSUMA

Scion Variety	Inoculum Source	Plants Inoculated	Plants with Abnormal Bud Union*
Valencia	Hayashi Satsuma	4	4
	Okitsu-Wase Satsuma	3	3
	Checks	3	0
Hassaku	Hayashi Satsuma	3	3
	Okitsu-Wase Satsuma	3	2
	Checks	3	0
Yuzu	Hayashi Satsuma	2	2
	Checks	2	0

\*Examined 1 year after bud inoculation.

TABLE 3  
INFECTIVITY OF BUDS TAKEN FROM *P. TRIFOLIATA* SEEDLINGS PREVIOUSLY INOCULATED BY BUDS OF BUD-UNION-CREATED SATSUMA TREES

Original Inoculum Source	Variety of Bud Inoculum	Plants Inoculated*	Plants Infected
Hayashi Satsuma	Inoculated <i>P. trifoliata</i> seedlings	2	0
	Infected Satsuma scions as checks	4	4
Okitsu-Wase Satsuma	Inoculated <i>P. trifoliata</i> seedlings	2	0
	Infected Satsuma scions as checks	3	3

\*Valencia orange on *P. trifoliata* rootstock.

TABLE 4  
COWPEAS DEVELOPING NECROSIS FOLLOWING INOCULATION WITH THE PATHOGEN ASSOCIATED WITH BUD-UNION DISORDER OF SATSUMA

Inoculum Source	No. Plants Affected / No. Plants Inoculated		
	Exp. 1	Exp. 2	Exp. 3
Hayashi Satsuma	8 / 13	9 / 11	3 / 6*
Okitsu-Wase Satsuma	11 / 12	2 / 6	2 / 6*
Noda Satsuma	8 / 10	—	—

\*Inocula for experiment 3 were from infected cowpea.

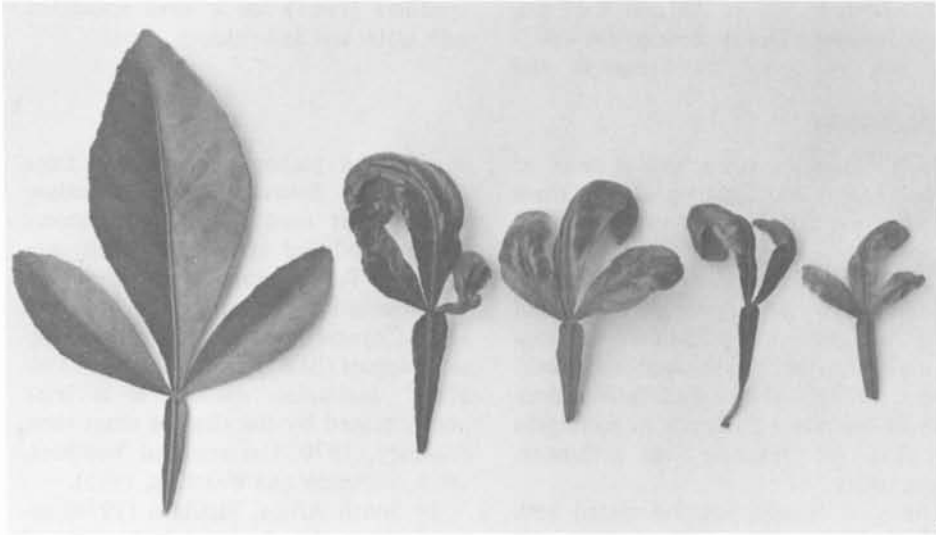


Fig. 3. Mottled and distorted leaves of citremon inoculated with Okitsu-Wase isolate.

TABLE 5  
DISTRIBUTION OF THE VIRUS PARTICLE  
SIZES OBTAINED FROM TROYER  
CITRANGE INOCULATED WITH  
BUD-UNION CREASE OF SATSUMA

Length (nm)	No. of Particles
550	7
615	32
670	167
700	10
715	2
Total	218

pea plants. About 10 days after inoculation, necrotic local lesions accompanied by vein necrosis developed on primary leaves inoculated singly from each of the three inoculum sources (table 4). The necrotic lesions were usually a spreading type and severe stem necrosis developed later (fig. 4). The symptoms on cowpea were similar to those described by Garnsey and Weathers (1968) on Early Ramshorn cowpea.

#### ELECTRON MICROSCOPY OF INFECTED CITRUS AND COWPEA

Dip preparations were obtained from infected cowpea leaves and stems, and from infected citrus leaves (table 5). Flex-



Fig. 4. Necrosis on cowpea inoculated with Okitsu-Wase isolate.

uous particles, 600 to 700 nm X 15 nm, were detected. This is close to the 650 X 19 nm reported by Semancik and

Weathers (1965) for a virus associated with tatter leaf and citrange stunt.

## DISCUSSION

The three inoculum source trees of bud-union-creased Satsuma used in these experiments had the same symptoms and were thought to have the same disease. Symptoms identical to those of the original inoculum source trees were obtained by serial inoculation of healthy budlings of Satsuma on *P. trifoliata* rootstock. This is strong evidence that the inoculum sources contain a pathogen or pathogens capable of inducing this bud-union abnormality.

Buds of *P. trifoliata* inoculated with infected buds of the bud-union creased Satsuma were free from the pathogen even when the inoculum buds still remained in the bark of the inoculated seedlings. This indicates that *P. trifoliata* is immune to the virus associated with the bud-union crease of Satsuma. However, the pathogen could pass through *P. trifoliata* tissue. Similar phenomena were reported for yellow vein and tristeza viruses (Weathers, 1961; Miyakawa, 1971; Tanaka *et al.*, 1971).

Calavan *et al.*, (1963) and Garnsey (1970) reported that the tatter leaf-citrange stunt virus complex caused bud-union crease in Satsuma and some other varieties on citrange and *P. trifoliata* root-

stocks. The pathogen transmitted from one of the diseased Satsuma inoculum source trees used in our experiments clearly produced symptoms of citrange stunt in Troyer citrange and citremon. The reaction of Valencia on *P. trifoliata*, that of cowpea, and electron microscopy also support the hypothesis that the "Dai-Make" bud-union disorder is at least partly caused by the citrange stunt virus (Garnsey, 1970; Garnsey and Weathers, 1968; Semancik and Weathers, 1965).

In South Africa, McClean (1974) reported that the abnormal bud union of sweet orange on rough lemon rootstock is caused by a transmissible pathogen. Bud-union abnormalities on the same scion-rootstock combination have also been reported from other countries (Bridges and Youtsey, 1968; Grant *et al.*, 1957; Grimm *et al.*, 1955). In a limited inoculation test to Valencia orange on rough lemon rootstock, the pathogen used in our experiments produced no symptoms on this scion-rootstock combination.

From the results obtained, it appears that the pathogen or pathogens causing the bud-union abnormality of Satsuma in Japan consists, at least partially, of tatter leaf-citrange stunt virus or a related virus.

## LITERATURE CITED

BRIDGES, G. D., AND C. O. YOUTSEY

1968. Further studies on the bud-union abnormality of rough lemon rootstocks with sweet orange scions, p. 236-39. In J. F. L. Childs, (ed.), Proc. 4th Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.

CALAVAN, E. C., D. W. CHRISTIANSEN, AND C. N. ROISTACHER

1963. Symptoms associated with tatter-leaf virus infection of Troyer citrange rootstocks. Plant Dis. Rep. 47:971-75.

GARNSEY, S. M.

1970. Viruses in Florida's 'Meyer' lemon trees and their effects on other citrus. Proc. Fla. State Hort. Soc. 83:66-71.

GARNSEY, S. M., AND L. G. WEATHERS

1968. Indexing for tatter leaf with cowpea, p. 80-82. In J. F. L. Childs, (chmn.), USDA Agr. Handbook No. 333, Washington.

- GRANT, T. J., S. MOREIRA, AND A. S. COSTA  
1957. Observations on abnormal citrus rootstock reactions in Brazil. *Plant Dis. Rep.* 41:743-48.
- GRIMM, G. R., T. J. GRANT, AND J. F. L. CHILDS  
1955. A bud union abnormality of rough lemon rootstock with sweet orange scions. *Plant Dis. Rep.* 39:810-11.
- McCLEAN, A. P. D.  
1974. Abnormal bud union between some sweet oranges and rough lemon rootstock: evidence of cause by a transmissible pathogen, p. 203-10. *In* L. G. Weathers and M. Cohen, (eds.), *Proc. 6th Conf. Intern. Organization Citrus Virol. Univ. California Div. Agr. Sci., Berkeley.*
- MIYAKAWA, T.  
1971. Symptoms in citrus varieties induced by the virus (tristeza stem pitting) associated with Hassaku dwarf disease. *Bul. Tokushima Hort. Exp. Sta.* 4:1-15.
- SEMANCIK, J. S., AND L. G. WEATHERS  
1965. Partial purification of mechanically transmissible virus associated with tatter leaf of citrus. *Phytopathology* 55:1354-58.
- TACHIKAWA, T., T. TSUCHIYA, I. IGUCHI, AND S. HARA  
1968. Investigations on the incompatibility between Okitsu-Wase Unshiu and its rootstock. *Shizuoka Pref. Citrus Exp. Sta. Bul. No. 7*, p. 1-11.
- TANAKA, H., S. YAMADA, AND J. NAKANISHI  
1971. Approach to eliminating tristeza virus from citrus trees by using trifoliate orange seedlings. *Bul. Hort. Res. Sta., Japan, Ser. B, No. 11*, p. 157-65.
- WALLACE, J. M., AND R. J. DRAKE  
1963. New information on symptom effects and host range of the citrus tatter-leaf virus. *Plant Dis. Rep.* 47:352-53.
- WEATHERS, L. G.  
1961. Responses of citrus to concurrent infection with two or more unrelated viruses, p. 187-96. *In* W. C. Price, (ed.), *Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.*