OTHER CITRUS VIRUS DISEASES

Occurrence and Varietal Distribution of Tatter Leaf-Citrange Stunt Virus and Its Effects on Japanese Citrus

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Tatter leaf disease of citrus was first described in California (Wallace and Drake, 1962) and later detected in a variety collection in Florida (Garnsey, 1964). Calavan *et al.* (1963) and Garnsey (1970) reported that a bud-union crease was induced in citrus trees on citrange and trifoliate orange rootstocks by inoculation with tatter leaf-infected citrus tissues. Later, a virus disease designated as citrange stunt was differentiated from tatter leaf (Wallace and Drake, 1968). Miyakawa and Matsui (1976) reported that satsuma mandarin trees grafted on trifoliate orange which had abnormal bud unions were infected with a virus or viruses similar to those causing tatter leaf and citrange stunt. This paper reports the results of indexing for tatter leaf-citrange stunt virus and its relation to bud-union crease symptoms in field-grown citrus trees grafted on trifoliate orange rootstock in Japan.

MATERIALS AND METHODS

Inoculum source trees. Inocula were collected from several districts in Japan. Buds collected from Ponkan and Tankan trees have grown normally on Yuzu or Shekwasha rootstocks. Buds from most source trees of two other varieties, satsuma (except three sources on Yuzu rootstock) and pummelo grafted on trifoliate orange have shown abnormalities such as basal scion swelling and crease at the bud union (fig. 1A, B). In addition, boat-shaped leaves similar to those of satsuma dwarf disease were often observed on the satsuma trees.

Indexing procedures for tatter-leaf virus. Buds of *C. excelsa* were grafted onto potted plants previously graft-inoculated by budding with material from suspect trees. These plants were kept at 22-24°C for 4-6 weeks. Spotting, irregular patterns and deformities of newly developed leaves of *C. excelsa* indicated tatter-leaf infection (fig. 2A).

Detection of citrange stunt virus and the bud-union crease agent. Seedlings of Troyer and Rusk citrange were bud-inoculated (Garnsey, 1964; Wallace and Drake, 1963), the tops were cut back to force new shoot growth and observed for symptoms for at least a year.

Virus-free budlings of sweet orange or satsuma mandarin on trifoliate orange were used to detect the bud-union crease agent. Bark of inoculated indicator plants was peeled at the bud union about a year after inoculation to reveal bud-union crease.

Mechanical inoculation of cowpea. New leaves were collected from citrus plants previously inoculated with the virus isolates or from subpropagations of infected trees. Sap inoculations were made on the primary leaves of Blackeye and Early Ramshorn cowpea (Garnsey and Weathers, 1968). Inoculated plants

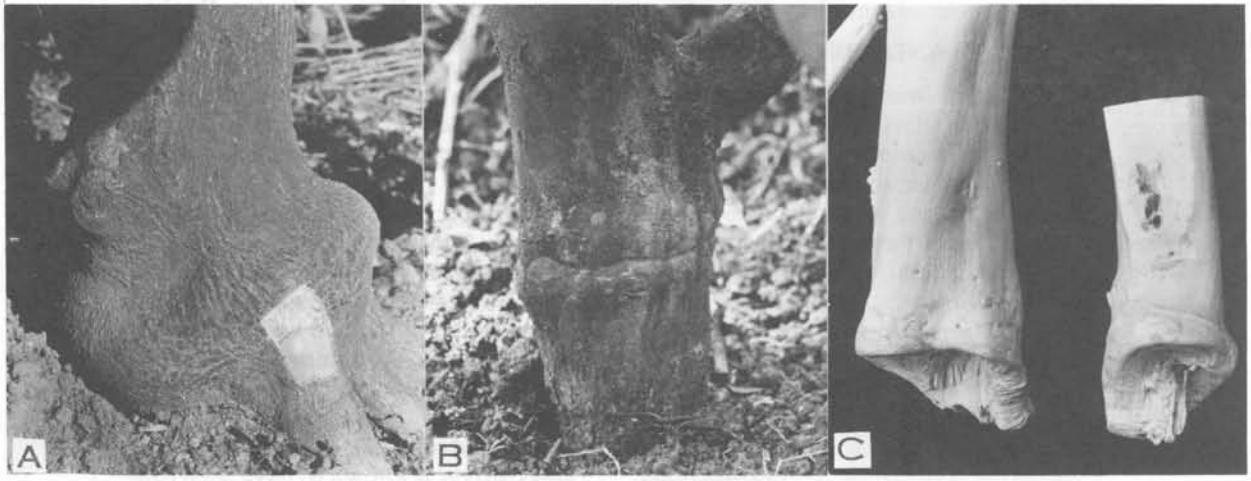

Fig. 1. Bud-union abnormalities of citrus on trifoliate orange. A) Basal scion swelling of a satsuma tree top-worked with ponkan buds several years ago (crease is present at the union), B) bud-union crease of pummelo (Suisho Buntan), C) Ponkan trunks broken at the bud union with trifoliate orange by strong winds.

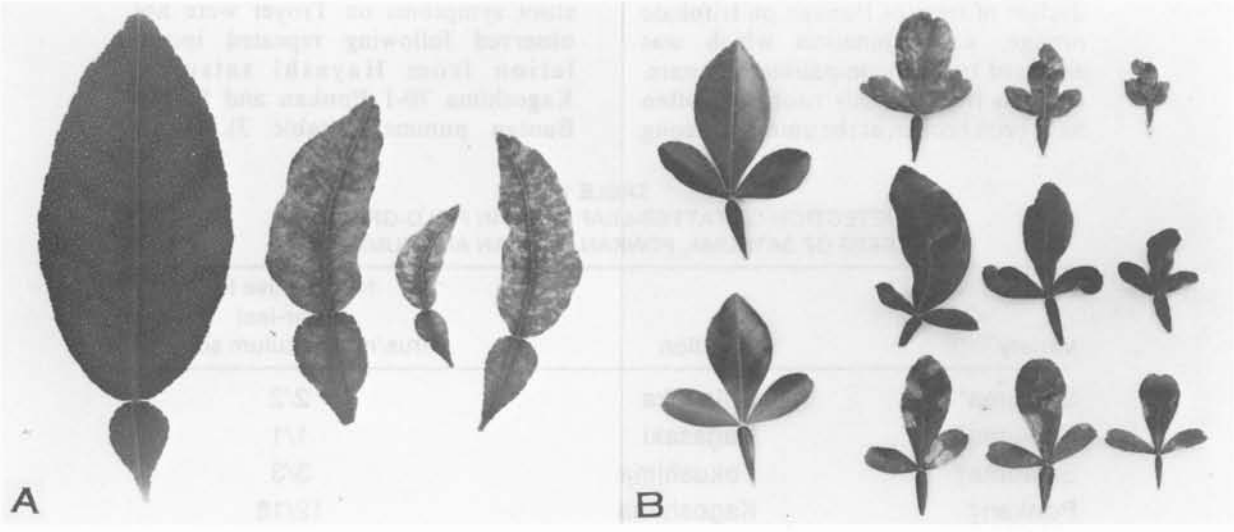

Fig. 2. Reactions of indicator plants. A) Tatter-leaf symptoms on *C. excelsa*, and B) various types of symptoms on Troyer citrange leaves inoculated from Okitsu-Wase satsuma. Healthy leaves at left.

were kept at 20-22°C for 2 weeks. Local lesions appeared 5-6 days after inoculation and vein and stem necrosis followed as symptoms advanced.

RESULTS AND DISCUSSION

All three inoculum sources from satsuma mandarin trees which showed bud-union crease on trifoliate orange and three other sources of satsuma on Yuzu rootstock which showed boat-shaped leaves were positive for tatter-leaf virus (table 1). The symptoms on *C. excelsa* were identical to those described previously (Wallace and Drake, 1962) (fig. 2A). One cultivar, Okitsu-Wase satsuma, is from a nucellar budline released for propagation in 1949 and was thought to be virus-free at that time. Trees from other sources of this budline still have normal bud unions on trifoliate orange. Some trees of this budline have apparently become infected with the virus, perhaps by top-working onto infected trees.

A high percentage of Ponkan budwood collected from Kagoshima, Kochi and Nagasaki was found infected with tatter-leaf virus. This may explain the decline of trees of Ponkan on trifoliate orange, a combination which was assumed to be incompatible for years. Ponkan trees on this rootstock often have been broken at the union by strong winds (fig. 1C). However, inocula from a few source trees of Ponkan caused no tatter-leaf symptoms or bud-union crease on indicator plants. Ponkan cultivars were introduced to Japan from China (mainly Taiwan) by budwood during the last 50 years. It is, therefore, conceivable that most introductions of this variety had been infected with the virus.

Inoculum from a pummelo cultivar (Suisho Buntan) in Kochi caused very mild tatter-leaf symptoms in *C. excelsa.* However, the inoculum source trees had clear creases at the bud unions (fig. 1B) and budlings of pummelo and some other varieties on trifoliate orange showed clear bud-union crease when inoculated (table 2). The original tree of this cultivar is known to have been top-worked on a Ponkan tree.

Several inoculum source trees indexed for tatter leaf on *C. excelsa* were also indexed for citrange stunt on Troyer and Rusk citrange. Troyer citrange consistently reacted to the isolate from Okitsu-Wase and TL100 (Riverside isolate provided by Dr. E. C. Calavan) (fig. 2B). However, citrange-stunt symptoms on Troyer were not observed following repeated inoculation from Hayashi satsuma, Kagoshima 70-1 Ponkan and Suisho Buntan pummelo (table 3). These

TABLE 1
DETECTION OF TATTER-LEAF VIRUS IN FIELD-GROWN TREES OF SATSUMA, PONKAN, TANKAN AND PUMMELO

Variety	Location	No. positive for tatter-leaf virus/no. inoculum sources
Satsuma*	Shizuoka	2/2
Satsuma*	Nagasaki	1/1
Satsuma†	Tokushima	3/3
Ponkan†	Kagoshima	12/18
Ponkan†	Kochi	7/8
Ponkan†	Nagasaki	1/1
Tankan	Kagoshima	2/2
Pummelo*	Kochi	1/1

* Grafted on trifoliate orange in the field and showing bud-union crease.
† Grown on Yuzu or Shekwasha rootstocks and having normal appearing bud unions.

isolates produced clear symptoms on Rusk citrange, indicating that development of citrange-stunt symptoms on Rusk citrange is more consistent than on Troyer.

Tatter leaf-affected satsuma trees in the field often had boat-shaped leaves resembling those associated with satsuma dwarf disease. Potted satsuma trees inoculated with the virus in the greenhouse also produced similar symptoms. However, the inoculum did not affect sesame, a herbaceous indicator for satsuma dwarf virus (Tanaka, 1968).

Blackeye and Early Ramshorn cowpeas were inoculated with several isolates using tissue from the original subpropagation, or from previously bud-inoculated budlings or seedlings. There was considerable variation in the reaction among the cowpea plants, even when the same citrus variety was used for inoculum. Most isolates tested produced symptoms on cowpea plants, however, two from Kagoshima 70-1 Ponkan and one from Suisho Buntan pummelo did not cause symptoms in repeated tests. From the existing evidence, I cannot determine whether different virus strains and components were present and further experiments will be required.

It appears that trees with bud-union crease are consistently infected with the virus causing tatter leaf on *C. excelsa* and citrange stunt on Rusk citrange (tables 2 and 3). I suggest, therefore,

TABLE 2
COMPARATIVE REACTIONS OF SEVERAL ISOLATES ON INDICATOR PLANTS FOR BUD-UNION CREASE AND TATTER LEAF

Isolate	Bud-union crease*	Tatter leaf† No. positive/no. inoc.
Okitsu-Wase satsuma	Positive	6/7
Noda satsuma	Positive	3/4
Hayashi satsuma	Positive	7/7
Ponkan (Kagoshima 70-1)	Positive	6/6
Pummelo (Suisho Buntan)	Positive	8/12
Meyer lemon (UCR TL100)	Positive	5/5

* Tested by inoculating potted budlings of sweet orange or satsuma on trifoliate orange rootstocks.
† Buds of *C. excelsa* were grafted to infected plants.

TABLE 3
CITRANGE-STUNT SYMPTOMS PRODUCED BY SEVERAL ISOLATES OF TATTER-LEAF VIRUS ON TROYER AND RUSK CITRANGES

Inoculum Source	Troyer No. positive/no. inoc.	Rusk No. positive/no. inoc.
Okitsu-Wase satsuma	10/12	3/3
Hayashi satsuma	0/11	7/8
Satsuma (Sakamoto)	0/2	2/2
Satsuma (Nakane)	0/4	2/2
Ponkan (Kagoshima 70-1)	1*/7	5/5
Pummelo (Suisho Buntan)	0/3	2/3
Check	0/5	0/3

* Slight.

that tatter leaf on *C. excelsa* and citrange stunt on Rusk citrange are the most reliable and useful symptoms for indexing the pathogen which induces bud-union crease.

The presence of severe seedling yellows tristeza, which is widespread in mandarin-type citrus varieties in Japan, has made it difficult to detect tatter-leaf virus in seedlings of *C. excelsa*, because these usually show severe chlorosis, yellowing and stunting when infected with seedling yellows tristeza virus. This difficulty was overcome by top-grafting *C. excelsa* or Rusk citrange buds on previously inoculated rough lemon plants rather than relying on direct inoculation of indicator seedlings (Miyakawa and Matsui, 1976).

ACKNOWLEDGMENT

I am grateful to Dr. E. C. Calavan and Mr. C. N. Roistacher, University of California, Riverside, for supplying experimental materials and reading the manuscript.

LITERATURE CITED

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.
1964. Detection of tatter leaf virus of citrus in Florida. Proc. Fla. State Hort. Soc. 77:106-09.

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 (ed.), Agric. Handb. 333, U.S. Dept. Agric., Wash., D.C.

MIYAKAWA, T., and C. MATSUI
1976. A bud-union abnormality of satsuma mandarin on *Poncirus trifoliata* rootstock in Japan. p. 125-31. *In* Proc. 7th Conf. IOCV. IOCV, Riverside.

TANAKA, S.
1968. Satsuma dwarf. p. 56-59. *In* J.F.L. Childs (ed.), Agric. Handb. 333, U.S. Dept. Agric., Wash., D.C.

WALLACE, J.M., and R.J. DRAKE
1962. Tatter leaf, a previously undescribed virus effect on citrus. Plant Dis. Rep. 46:211-12.

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.

WALLACE, J.M., and R.J. DRAKE
1968. Citrange stunt and ringspot, two previously undescribed virus diseases of citrus. p. 177-83. *In* Proc. 4th Conf. IOCV. Univ. of Florida, Gainesville.