

Occurrence and Distribution of Citrus Tatter Leaf in Fujian, China

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ABSTRACT. In Fujian, Ponkan, Fuju and Satsuma mandarin and Sekkan sweet orange are the most widely planted cultivars in commercial orchards. Recently, however, symptoms of swelling, budunion crease and incompatibility have been observed on some field trees of trifoliolate rootstock budded with Ponkan, Sekkan and Bendizao.

Results of detection by using Kalpi lime, Troyer and Rusk citrange and cowpea as indicator plants showed that the bud union symptoms were caused by the citrus tatter leaf virus (CTLV) in Fujian citrus producing areas. The other cultivars infected with CTLV were Beijing lemon and Bainianju, Fuju, and Satsuma mandarin. The disease was distributed in nine counties of Fujian province, i.e. Jiayang, Jian'ou, Fu'an, Shaxian, Sanming, Yong'an, Longyan, Yongchun and Changtai.

The CTLV has been partially purified and particles of different sizes, 700-850 x 15 nm and 400-500 x 10 nm, have been obtained from different isolates.

Tatter leaf was discovered for the first time in 1962 on Beijing (Meyer) lemon trees which had been introduced from China to California, USA (11). Afterwards, this disease was found in Florida (USA) (4), Japan (6), Australia (3), and South Africa (2). Recently, the occurrence of the disease and its damage to citrus have been reported in Taiwan (10), Zhejiang (14), Sichuan (13) and Hunan (Shu Guangping, personal communication). It attacks mainly citrus cultivars grafted on trifoliolate (or trifoliolate hybrids) rootstock, causing budunion crease, yellow ring groove, yellowing and decline of canopy (6, 8, 13, 14). In addition, many varieties on non-trifoliolate orange rootstocks carry the tatter leaf virus although they show no symptoms. The virus particle is a flexuous rod, 600-700 x 15 nm or 650 x 19 nm (6, 9) or 450-900 nm (14). Kalpi lime, Troyer and Rusk citrange, citremon and cowpea have been commonly chosen as the indicator plants for the disease (1, 5, 6, 12). The causal virus is complex as it has at least 2-3 components depending on various symptoms shown on different index plants (7, 8).

This disease probably originated in China (6, 10, 14) and was distributed to the leading citrus producing areas, and is a potential hazard to commercial production of citrus. A survey for the disease has been car-

ried out in the citrus areas of Fujian since 1985 and is reported in this article.

MATERIALS AND METHODS

Survey method. Our survey was carried out in the citrus orchards of 23 counties. The growth of cultivars was observed, including Ponkan, Fuju (mandarin), Sekkan (orange), Bendizao (*C. succesa*), Beijing lemon (*C. limon* x *C. sinensis*?) which had been grafted on trifoliolate orange or other rootstocks; and special attention was paid to occurrence and severity of budunion crease, yellow ring groove and yellowing of canopy.

Indexing procedure. Budwood or leaves were chosen as the inoculation source and were taken from the above mentioned citrus cultivars from different places and Kalpi lime, Troyer and Rusk citrange were inoculated by grafting and cowpea was inoculated by rubbing. The isolate from local lesions of cowpea was inoculated to Kalpi lime and Troyer citrange by razor-cut inoculation of stems. HN-B (from the diseased Bingtong orange, supplied by Hunan Academy of Agricultural Sciences) and ZJ-T (from the Troyer citrange, supplied by Taizhou Agricultural School of Zhejiang) were chosen as positive controls. Plants were kept in a screenhouse.

Partial purification of the virus. Isolates SM-P (from a diseased Ponkan tree in Sanmin) and LY-BM (from the latent infected Beijing lemon tree in Longyan) maintained on Troyer citrange were used as the virus source for purification. Fifty grams of diseased leaves at -60°C were cut into tiny pieces to which was then added by 250 ml of 0.1 M Tris-HCl buffer (containing 0.15% (v/v) Triton X-100), pH 8.0 and 2.5 g bentonite. It was filtered and centrifuged at 10,000 g for 2 min. The supernatant was concentrated by two cycles of PEG-6000 precipitation. The pellet was suspended in a small quantity of 0.05 M Tris-HCl buffer, pH 8.0, at 0°C for 1 hr and centrifuged at 5,000 g for 10 min. The supernatant was fractionated by non-continuous sucrose density-gradient centrifugation (200 mg/ml, 350 mg/ml, 450 mg/ml, 650 mg/ml, prepared with Tris-HCl buffer) in Hitachi RPS 40T Rotor and centrifuged at 30,000 rpm for 3 hr. The position between 450 mg/ml and 650

mg/ml with the virus band was collected, diluted and centrifuged in RP 42 Rotor at 40,000 rpm for 2 hr. The pellet was suspended in several drops of Tris-HCl buffer. This preparation was considered partially purified virus.

Electron microscopy. Samples were made with the preparation of partially purified virus from young diseased citrus leaves and cowpea leaves with necrotic lesions. They were observed under a JEM-100 CXII electron microscope after negative staining with uranyl-acetate.

RESULTS

A field survey on citrus tatter leaf in Fujian. Some citrus trees on trifoliate rootstock showed symptoms of swelling, crease and incompatibility at budunion causing a separation layer (Fig. 1). Thus, it was broken readily at the budunion by strong winds. The disease trees also showed stunting and leaf yellowing of the



Fig. 1. A bud union crease with yellow ring groove (left) and yellowing canopy (right) in Ponkan grown on trifoliate orange rootstock.

canopy with debilitated appearance (Fig. 1). The citrus orchard incurred a great economic loss. The above-mentioned symptoms, which were found in the citrus areas of Longyan, Yang'an, Sanmin, Shaxian, Jianyang, Jian'ou, Fu'an, Yongchun and Changtai counties, were very similar to those of tatter leaf although the incidence of the disease varied from 1% to 70% from place to place.

In Fujian the major citrus cultivars are Ponkan, Fuju, Sekkan and Satsuma, whereas Tankan, Bendizao and lemon rank next. Our field survey indicated that diseased trees of Ponkan, Sekkan and Bendizao grafted on trifoliolate rootstock showed typical symptoms, whereas Fuju and lemon on trifoliolate rootstock and all the cultivars on Fuju rootstock did not display obvious symptoms. However, some citrus trees with a healthy appearance have been shown to carry the tatter leaf virus.

Detection of tatter leaf virus. All budwood taken from seven diseased trees of Ponkan, Sekkan and Bendizao on trifoliolate orange rootstock and the budwood from 21 healthy appearing trees of Fuju, Bainianju (mandarin), Xinhui (orange), Tankan and Beijing lemon on Fuju rootstock were positive for CTLV on Kalpi lime, Troyer and Rusk citrange. And these inoculated indicator plants showed typical symptoms of stunting, small leaves, tatter leaf and yellow blotch (Fig. 2). The latent period ranged from 45 days to one year. Moreover, new shoots growing from the diseased shoots of Kalpi lime generally showed no symptoms, but carried the virus. It could be detected by rubbing the sap of diseased young leaves on to cowpea, which produced necrotic local lesions on the inoculated leaves 3-4 days after inoculation. By comparing the symptoms of indicator plants with typical symptoms caused by the two positive controls (HN-B and ZJ-T), it was confirmed that tested citrus trees had been infected by CTLV.

During the detection of CTLV, seven isolates from seven citrus cultivars: Beijing lemon (LY-BM), Xinhui orange (XH-O), Bainianju (YC-BNJ), Ponkan (SM-P), Ponkan (CT-P), Tankan (CA-T) and Fuju (JO-FJ) and one isolate from cowpea (YC-P) showed different infectivities which could be classified into three types (Table 1). Type A, represented by SM-P, could infect Kalpi lime, but could not infect cowpea, and it showed a latent infection without symptoms on Troyer or Rusk citranges; the virus was detected on Kalpi lime. Type B, represented by LY-BM, could infect Kalpi lime, citrange and cowpea. It is similar to the two positive controls HN-B and ZJ-T. Type C, represented by YC-P, could infect citrange by razor-cut inoculation and infect cowpea by sap inoculation, but did not infect Kalpi lime.

Electron microscopy. Under an electron microscope, a large number of flexuous rods, 700-850 x 15 nm (Fig. 3), similar to the 600-700 x 15 nm previously reported by Miyakawa (6), could be seen on the samples prepared with the extract from cowpea leaf with necrotic local lesions which had been inoculated with isolate YC-P and JO-FJ, respectively. Virus particles, 400-500 x 10 nm (Fig. 3), shorter and thinner, could be seen in the samples prepared with extract of Kalpi lime leaf inoculated by isolate SM-P. By using partial purification, a large number of virus particles 400-500 x 10 nm from SM-P infected Troyer citrange leaves and particles 700-850 x 15 nm from LY-BM infected Troyer citrange leaves could be seen under an electron microscope. The morphology and size of the virus particle from partial purification is similar to those in the extracts by dip-method.

DISCUSSION

In view of the bud union crease and canopy yellowing on field trees and the symptoms on Kalpi lime, the two citranges and cowpea, we consider this disease to be tatter leaf.

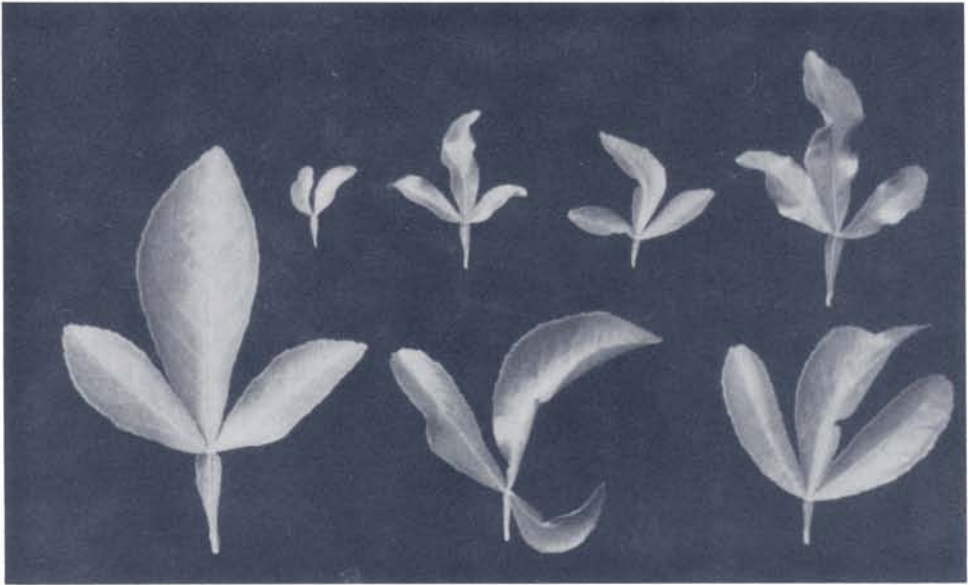
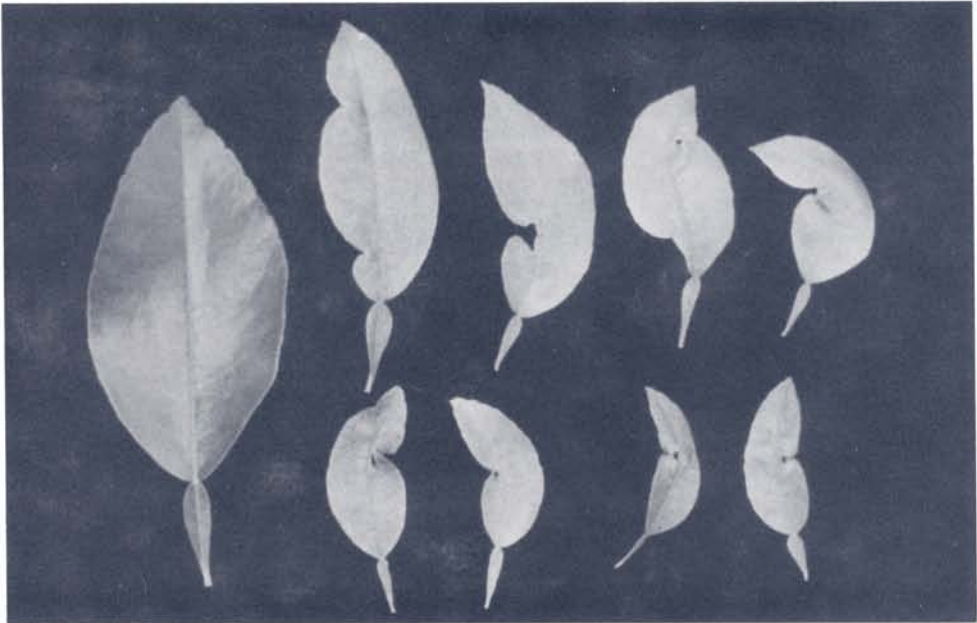


Fig. 2. Tatter leaf symptoms on *Citrus excelsa* (Top) and Rusk citrange (Bottom). Healthy control leaf at the left in each photo.

According to field survey and the detection by indicator plants, tatter leaf has occurred sporadically in nine counties of our province and is serious

in those areas that have introduced nursery trees from Zhejiang province. The disease mostly infects Ponkan, Sekkan, and Bendizao. Since

TABLE 1
A COMPARISON OF THE INFECTIVITY OF DIFFERENT ISOLATES
OF CITRUS TATTER LEAF VIRUS ON INDICATOR PLANTS

Isolate	Location	Indicator plants			
		Kalpi lime	Troyer citrange	Rusk citrange	Cowpea
A(SM-P)	Sanmin	2/5	0/5 ^y	0/6 ^y	0/62
B(LY-BM)	Longyan	2/5	0/5	2/5	2/30
HN-B ^z	Hunan	2/2	3/4	3/8	9/20
ZJ-T ^z	Zhejiang	2/4	4/7	5/7	6/20
C(YC-P)	Yongchun	0/5	2/5	—	3/20

^zPositive control

^yLatent infection

Ponkan is a principal commercial cultivar for export, the disease will probably become a new threat to the development of the citrus industry in our province.

Since tatter leaf virus has different components (7, 8), three isolates were obtained in our study. Isolate A (SM-P) can cause the typical symptoms only on Kalpi lime. Isolate B (LY-BM) can cause the typical symptoms on Kalpi lime, citrange and cowpea. Therefore, in order to avoid missing infected plants, it is suggested that the above three indicator plants be used simultaneously for detection of CTLV.

Virus particles 700-850 x 15 nm can be obtained from the isolate B (LY-BM) by means of a partial purification. Their size is close to that of the virus particles of tatter leaf reported by Miyakawa and Matsui (6), whereas the particles of 400-500 x 10 nm, obtained from the isolate A (SM-P) are different from those reported here and abroad.

Trifoliolate orange is an excellent rootstock for the major citrus cultivars in our province because it cannot only bring about early fruiting and high production, but resists cold, tristeza and foot-rot diseases. Over the recent years, some have suggested replacing trifoliolate orange with other rootstocks because of its susceptibility to exocortis and tatter

leaf. However, no insect vector has been found to transmit tatter leaf and exocortis and citrus cultivars grafted on non-trifoliolate rootstocks will come into production 1-2 yr later. Thus, replacement of trifoliolate rootstock will result in a great economic loss to citrus industry. It is recommended to produce disease-free nursery trees on trifoliolate rootstock to prevent spread of CTLV or other virus or virus-like diseases.

Although Fujou (mandarin) can be infected by tatter leaf on trifoliolate rootstock it shows no obvious symptoms, still grows well and shows no tendency to decline. When tatter leaf occurs on a Ponkan tree on trifoliolate orange rootstock, citrus growers of our province usually replace its rootstock by inarching two Fujou seedlings. The yellowing symptoms on the canopy will disappear on the Ponkan tree on the new rootstock one year later and it will grow normally. Those diseased trees whose trifoliolate orange rootstock has not been replaced by Fujou, decline considerably and eventually die. Obviously, Fujou is quite resistant to tatter leaf. However, it should be further studied whether Fujou can replace the trifoliolate rootstock to control the disease because the trees with new rootstock which are still carrying CTLV will probably remain a potential source of the disease.

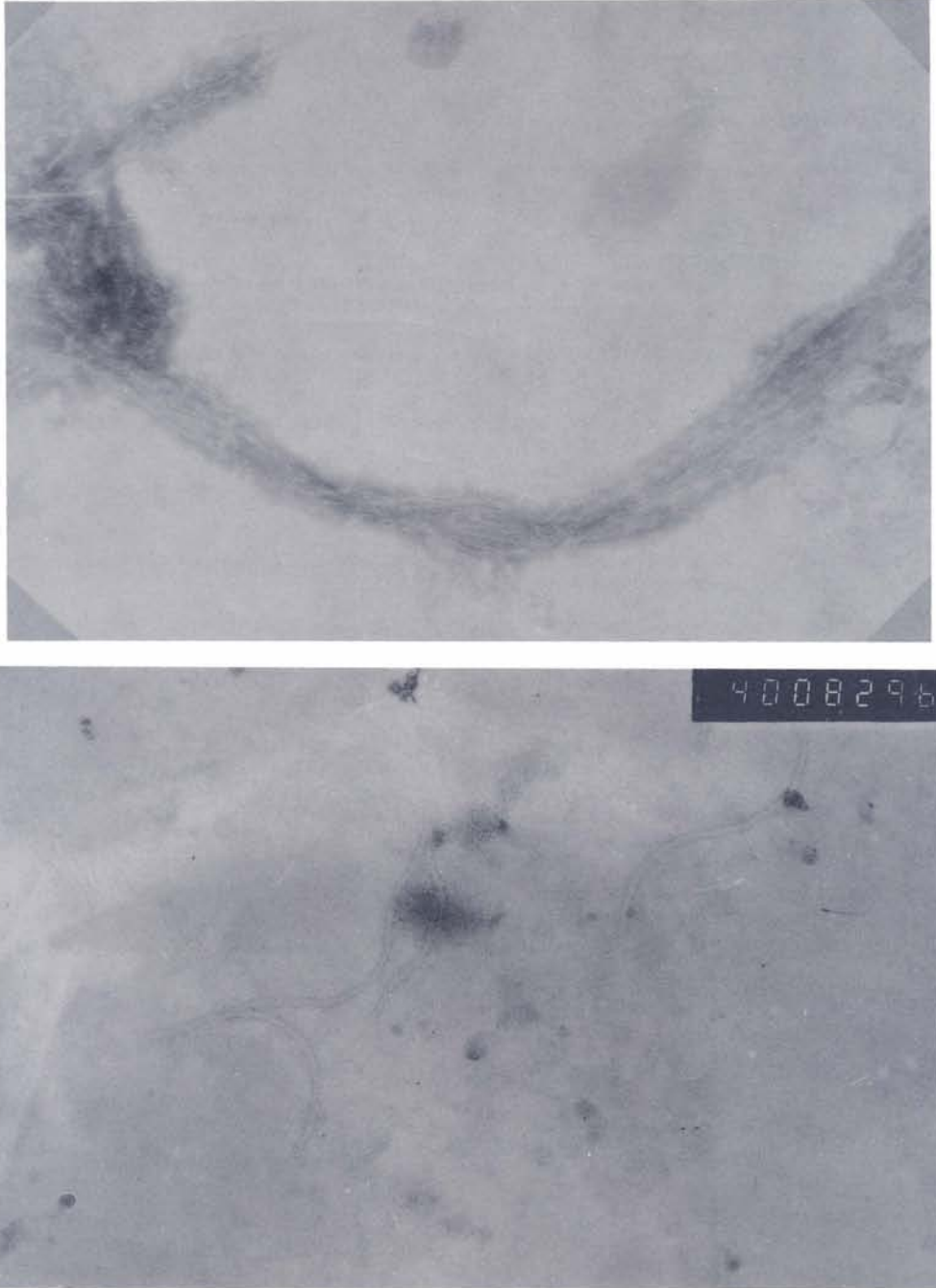


Fig. 3. Citrus tatter leaf particles of different sizes: 700-850 x 15 nm of LY-BM isolate (Top, 20,000 X) and 400-500 x 10 nm of SM-P isolate (Bottom, 100,000 X).

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