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Distribution of Citrus Tristeza Virus Antigen in Citrus Tissues

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Costa *et al.* (1949) indicated that citrus tristeza virus (CTV) was phloem-limited, and electron microscopic studies have supported that thesis (Kitajima and Costa, 1968; Shikata and Sasaki, 1969; Chen *et al.*, 1971). The CTV antigen was easily detected in phloem cells of various parts of citrus plants by the fluorescent antibody technique (Tsuchizaki *et al.*, 1978; Sasaki *et al.*, 1978). On the other hand, Schneider (1957, 1969) suggested that CTV was present in meristematic cells as well as in phloem.

The present paper reports the results of additional investigations of the distribution of CTV in citrus tissues in relation to strain severity and host susceptibility using the fluorescent antibody technique.

MATERIALS AND METHODS

Young and mature shoots were collected at various times of the year from field-grown or small, potted plants of Hassaku, Yuzu, and satsuma mandarin known to be infected with mild or severe strains of CTV. Petioles or stems were sectioned and stained as previously described (Sasaki *et al.*, 1978). Fluorescent antibody against CTV prepared by Tsuchizaki *et al.* (1978) was used in this investigation.

RESULTS AND CONCLUSIONS

The CTV antigen was detectable by the fluorescent antibody technique even in young shoots, 3 to 5 mm long, and cells containing the antigen reach a maximum 3 to 4 weeks after shoot initiation (Sasaki *et al.*, 1978). At that stage, regardless of the severity of the virus strain or the susceptibility of the host, cells containing viral antigen were present mostly in protophloem, and those in the metaphloem were few and scattered. This indicates that CTV multiplies most in actively-dividing cells. In Hassaku and Yuzu infected

with severe strains, the viral antigen was detected in a high percentage of cells in the ground meristem adjacent to protophloem (fig. 1). These cells failed to differentiate into cortex, or into rib tissue of the petiole. This concurs with Schneider's observations (1957) of chromatic cells in the ground meristem as well as in phloem.

On the other hand, the viral antigen was never seen in the ground meristem of Hassaku and Yuzu infected with mild strains, or in satsuma mandarin infected with mild or severe strains. Thus, even the severe strain of CTV is almost always restricted to phloem in varieties resistant to stem pitting, but invades to some extent the ground meristem of highly susceptible varieties, such as Hassaku and Yuzu, perhaps just before the phloem differentiates. In contrast, mild strains apparently do not move into the ground meristem regardless of host susceptibility.

In mature, 3-month-old shoots, the cells with CTV-specific fluorescence were rarely seen either in nonfunctional primary phloem or in cortical or rib tissue of petioles, but were always present in secondary phloem irrespective of the virus strain or of the host plant examined (fig. 2). Apparently, CTV particles degenerate almost completely within 3 months after synthesis, at least in the primary phloem or ground meristem of the shoot.

In primary phloem and cortical or rib tissue of petioles of Hassaku and Yuzu infected with the severe strains, some cells become necrotic (fig. 2). Such necrosis, however, was never seen in the same varieties carrying the mild strains, nor in satsuma mandarin infected with mild or severe strains. The response of infected cells to CTV is assumed to differ depending on strain severity and on host susceptibility.

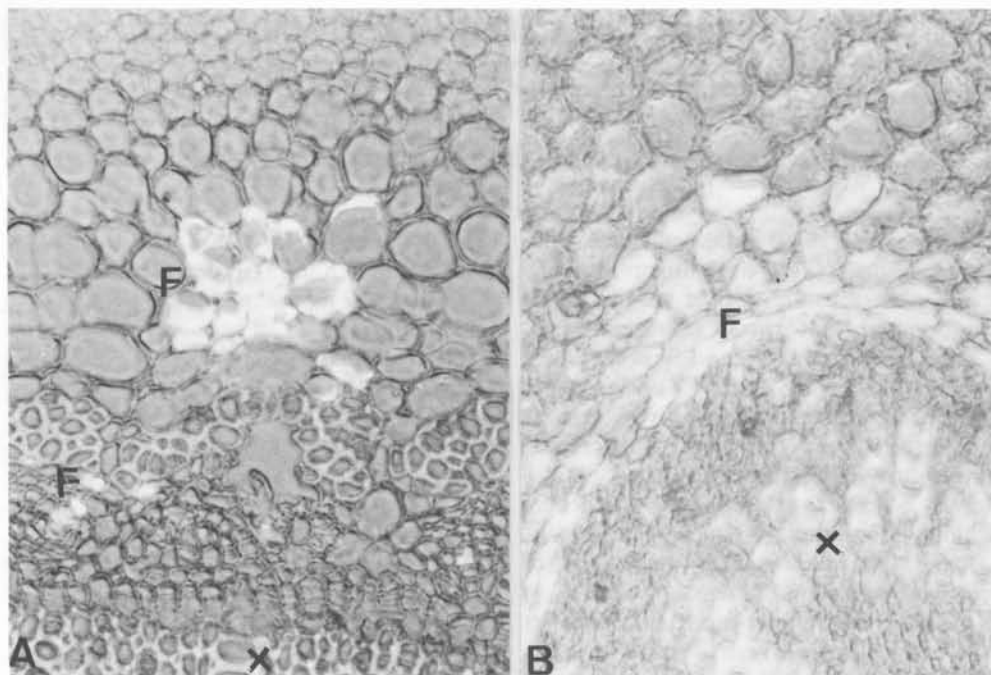


Fig. 1. Fluorescent plus phase contrast microscopy of sections of stem or petiole collected from citrus plants infected with severe strains of CTV. A) stem of 30-day-old shoot of Hassaku, B) petiole of about 14-day-old shoot of Yuzu. F, CTV-specific fluorescence; X, xylem. (400).

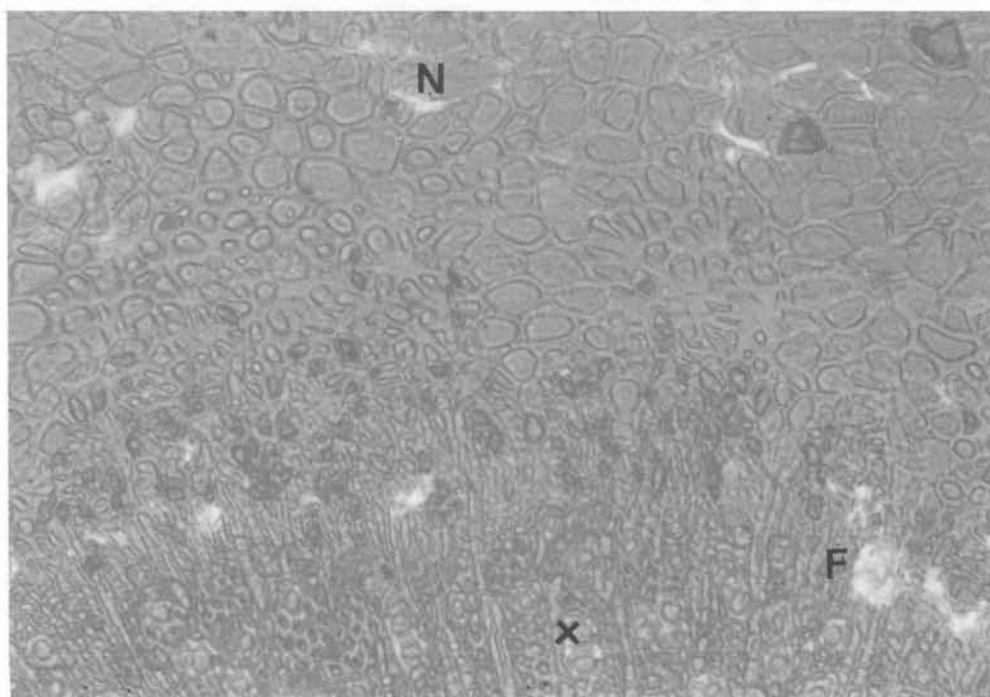


Fig. 2. Fluorescent plus phase contrast microscopy of a section of 90-day-old petiole from Hassaku infected with a severe strain of CTV. N, necrosis; F, CTV-specific fluorescence; X, xylem. (400).

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RESULTS

The appearance of post-treatment lesions was evaluated after 2, 3 and 4 weeks (Table I). Mildly necrotic lesions appeared very soon after inoculation with the tolerant strain No. 14 was very susceptible. Differences in the host tolerance among the 10 tolerant varieties were difficult to detect, but Yuzu-mikan and Tachibana appeared slightly tolerant. At 2°C infection and 27°C night-time temperature, host tolerance was related more to root system than to treatment variety. Tolerant varieties had rootstock graft union even before host treatment.

After 3 weeks the characteristic fluorescent bodies in the pericycle of stem and Citrus No. 14 stem disappeared, and the number of fluorescent bodies in the stem was reduced (Table I). However, from host-treated stem was raised on this host variety and CTV-free grafts of this variety and CTV-free grafts of tolerant variety were obtained. After 7 weeks characteristic fluorescent bodies in the pericycle of stem

disappeared in tolerant varieties. In the tolerant variety, CTV-free grafts were obtained in the first step toward the purpose.

MATERIALS AND METHODS

Two-year-old trees of 10 tolerant varieties (Tachibana, Yuzu, Yuzu-mikan, Yuzu-awase, Mexican lime, Tachibana, Yuzu-awase, Yuzu-awase, Yuzu-awase, Yuzu-awase, Yuzu-awase) and four tolerant varieties (Tachibana, Yuzu, Yuzu-mikan, Yuzu-awase) and Citrus No. 14 (Miyagawa-wase & Tamagiri) were planted in pots. Four trees were planted in each pot. The tolerant varieties were treated with CTV-free grafts before host treatment was started on a normally light photoperiod regime at 27°C daytime and 22°C night-time temperature and were kept at 27°C day and night.

After host treatment for 2, 3 and 4