Tristeza Virus in Citrus reticulata and C. tankan

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ABSTRACT

Long flexuous particles 2,000 × 12-14 mμ were observed in dip specimens prepared from veinal tissues of Likubin-diseased *Citrus reticulata* or *C. tankan*. Electron microscopy of ultrathin sections from diseased plants showed long flexuous particles restricted to the phloem cell. These intracellular filamentous particles were about 10 mμ in width, and most of them appeared as fibrous masses. In some cells, the filamentous particles were scattered randomly throughout the cells. Although the intracellular concentration of these filamentous particles was rather high, the phloem cells containing the filamentous particles were small in number. It was considered that the long flexuous particles observed in the present study corresponded to those described for citrus tristeza virus. It is not clear whether Likubin-diseased plants contain more than one agent or not. Phytopathology 61:279-282.

In Taiwan (Formosa), Likubin (Huanglungpin) is the most important disease in Ponkan (*Citrus reticulata* Blanco) and Tankan (*Citrus tankan* Hayata) production. According to the Mexican lime tests and transmission tests by aphids, Matsumoto & Su (6) considered that the causal agent of Likubin was closely related to citrus tristeza virus; however, they considered recently that Likubin was due to an unknown virus component in addition to citrus tristeza virus (10). Long filamentous virus particles about 2,000 × 10-12 mμ were observed first by Kitajima et al. (5) to be associated with citrus tristeza disease. Chen et al. (2) ascertained that similar particles were associated with dwarf disease of *Citrus hassaku*. Thin sectioning by Price (7, 8) and by Shikata & Sasaki (9) showed that these filamentous virus particles were restricted within the phloem cells of tristeza-diseased citrus plants. The characteristic symptoms of vein-clearing and stem-pitting appeared commonly on Mexican lime seedlings bud-grafted with Likubin-diseased Ponkan or Tankan. If the symptoms were induced by the citrus tristeza virus, then long filamentous particles should be encountered within Likubin-diseased citrus plants.

This paper deals with electron microscopy of citrus tristeza virus associated with Likubin in *C. reticulata* and *C. tankan*.

MATERIALS AND METHODS.—Young leaves of Ponkan and Tankan were collected for our study from the orchard of the National Taiwan University. In the orchard, the agent responsible for Likubin was transferred successively from plant to plant by grafting.

According to the method of Brandes & Bercks (1), leaf-dip specimens were prepared from leaf veins and interveinal mesophyll tissues of Ponkan showing vein-clearing and mottling.

For thin sectioning, pieces of leaves showing vein-clearing and mottling were fixed with paraformaldehyde-glutaraldehyde solution (4) for 1.5 hr. They were postfixed with 2% OsO_4 in phosphate buffer pH 7.4 for 3 hr. After dehydration, they were embedded in Epon 812. Thin sections were stained with uranium and lead.

RESULTS AND DISCUSSION.—Viruslike particles in leaf-dip specimens prepared from interveinal mesophyll tissues were not detected. On the other hand, long flexuous particles were observed in 3 leaf-dip specimens out of 20 prepared from the veins showing vein-clearing. The long flexuous particles were about 12-14 mμ in width. Although they were not uniform in length, some of them were about 2,000 mμ. Since their characteristic appearance (Fig. 1) was similar to that reported by Kitajima et al. (5), it was considered that these filamentous particles corresponded to citrus tristeza virus particles. Filamentous particles in leaf-dip specimens were rarely encountered; it was not clear whether intracellular concn of these filamentous particles was low or whether cells containing the filamentous particles were few in number. No filamentous particles were found in the dip specimens prepared from healthy leaves.

Filamentous particles similar to those found in leaf-dip preparations were not found in thin sections prepared from healthy veinal, interveinal mesophyll tissue or from interveinal mesophyll tissue from diseased plants.

In some thin sections prepared from the diseased veinal tissues, electron-dense substances were encountered in the cells of phloem tissues. A representative view observed at lower magnification is shown in Fig. 2. At higher magnification (Fig. 3), these substances were resolved into fibrous masses of long flexuous particles about 10 mμ wide. Another intracellular profile of the filamentous particles is shown in Fig. 4. In these cells, individual 10 mμ-wide particles are scattered randomly throughout the cells. Sometimes, masses of fine dots were intermingled with the filamentous particles as shown in Fig. 5. Since they were smaller than the ribosomes and their diam was about 10 mμ, we considered that these fine dots corresponded to the profiles of the filamentous particles cut in cross section or obliquely to their long axis. The long flexuous particles about 10 mμ in width were restricted within the cytoplasm of the phloem cells. In addition to the filamentous particles, tubular structures about 15 mμ in width were encountered frequently, as shown in Fig. 6 and 7. Presumably, these tubular structures corresponded to protein component (3). Although the tubular structures were encountered in both the diseased and healthy

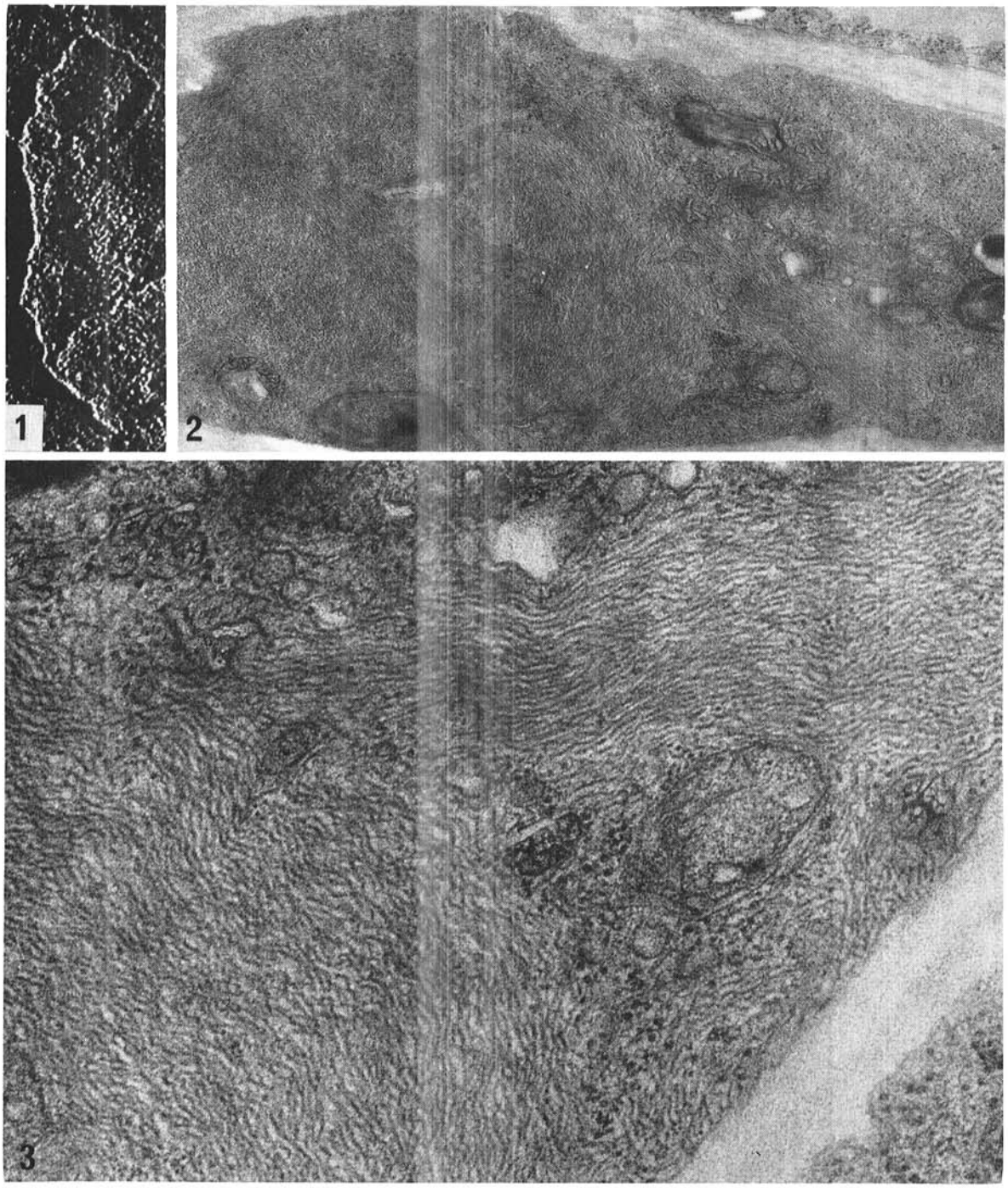

Fig. 1-3. **1)** Long flexuous particle in dip specimen prepared from diseased veinal tissue of *Citrus reticulata*. (×49,000) **2)** General view of the phloem cell *C. tankan* containing the long flexuous particles. (×27,000) **3)** Fine structural details of the long flexuous particles within the phloem cell (*C. tankan*). (×79,000)

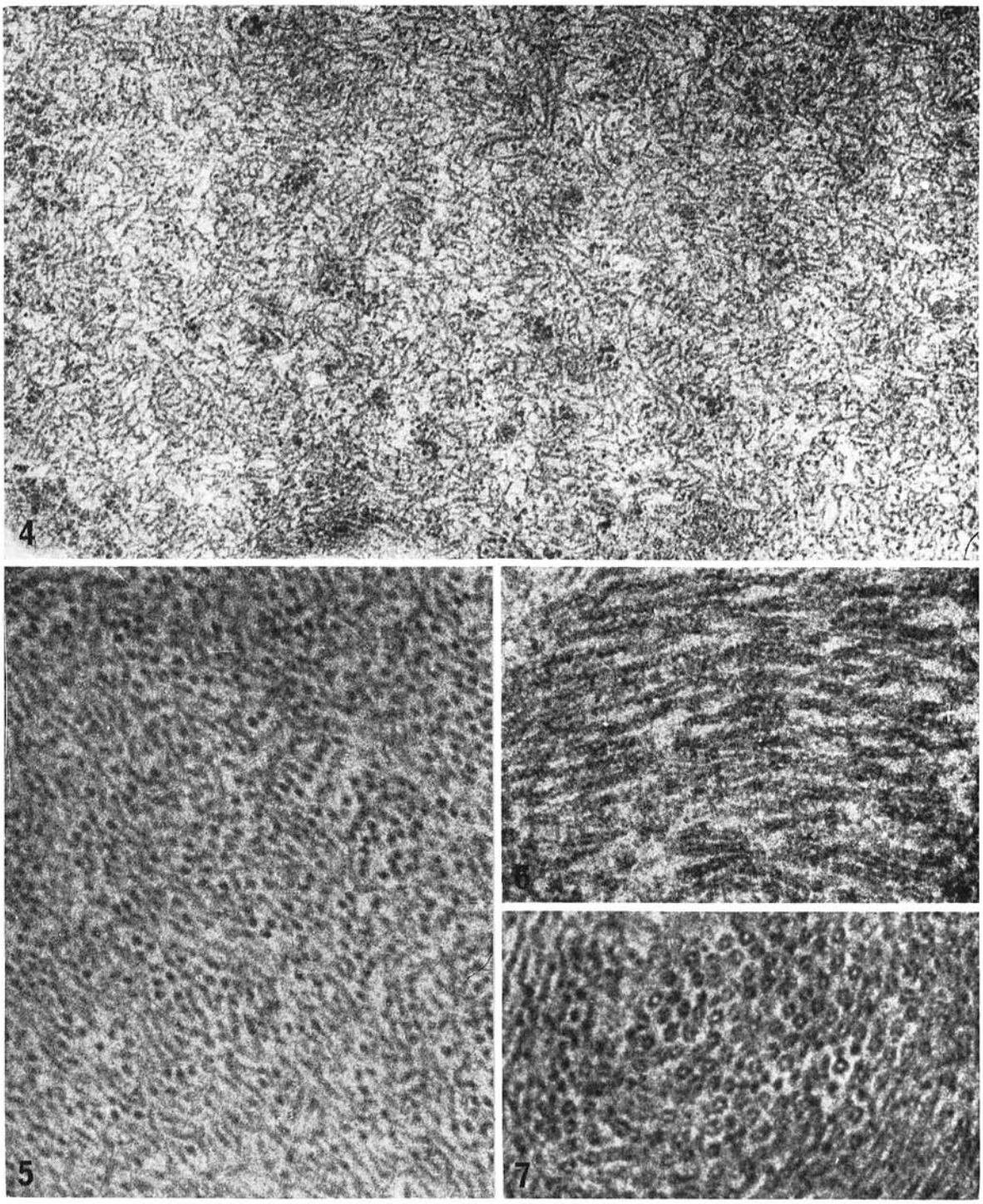

Fig. 4-7. 4) Long flexuous particles scattered randomly throughout the cell (*Citrus tankan*). (×60,000) **5)** Long flexuous particles cut in cross section or obliquely to their long axis. (×140,000) **6)** Mass of tubular structures. (×100,000) **7)** The tubular structures cut in cross section or obliquely to their long axis. (×190,000)

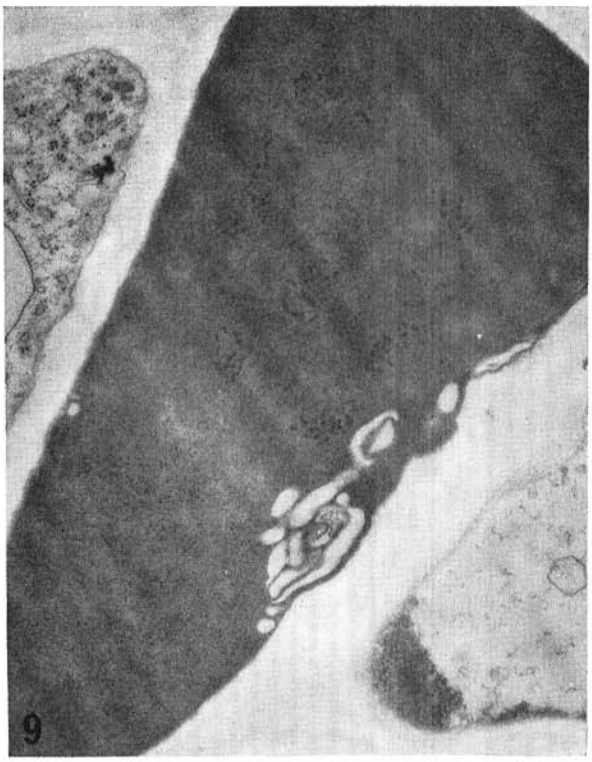

Fig. 8-9. 8) The cell (*Citrus tankan*) occupied completely by the long flexuous particles. (×48,000) **9)** Necrotic cells in the phloem tissue of diseased leaf. (×18,000)

phloem cells, they were encountered more frequently in the diseased phloem cells. Shikata & Sasaki (9) detected similar tubular structures within dwarf-diseased cells. Since the widths of intracellular long flexuous particles were close to those in dip specimens prepared from the diseased veinal tissues, and their general profiles were similar to citrus tristeza virus particles reported by Price (7, 8) and Shikata & Sasaki (9), we considered that these long flexuous particles corresponded to intracellular citrus tristeza virus particles. It is likely that the differences in width between the particles in the dip specimens and those in the cells resulted from shadowing procedures with chromium.

Although the cells containing the filamentous particles were few in number, large numbers of filamentous particles occurred within the cells. Moreover, as shown in Fig. 8, some cells were completely occupied by the filamentous particles. Accordingly, the rare appearance of filamentous particles in dip specimens probably was a result of the small number of cells containing the filamentous particles, not from the lower concn of the filamentous particles within the cells.

In the diseased phloem tissues, necrotic cells were frequently encountered as shown in Fig. 9. These cells were so dense that their intracellular fine structural detail was indiscernible, and it was not clear that these necrotic cells contained the filamentous particles. Stem pitting frequently associated with citrus tristeza virus infection may originate from these necrotic cells in the phloem tissues.

We encountered citrus tristeza virus frequently within Likubin-diseased Ponkan or Tankan. Accordingly, investigations concerning Likubin-diseased plants should be made in the absence of citrus tristeza virus infection.

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