# Electron Microscopy of the Tristeza Virus in Citrus Leaf Tissues

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IN RECENT STUDIES of leaf dip and partially purified preparations of tristeza with the electron miscroscope, thread-like particles, 10-12 mμ x 2,000 mμ were found (3) which are considered to be the causal virus. Subsequent studies of infected leaves revealed particles of similar dimensions in the phloem tissues.

## *Materials and Methods*

Galego lime [*Citrus aurantifolia* (Christm.) Swing.], Ruby Red grapefruit (*C. paradisi* Macf.), and Pera sweet orange [*C. sinensis* (L.) Osb.] seedlings were inoculated by grafting or by aphids, with mild or severe isolates of the tristeza virus under study in this department. About 2 months after inoculation, leaves were collected from these plants and fixed for 12-18 hr in cold 2 per cent osmium tetroxide in 0.2 M sodium phosphate buffer (5), dehydrated in acetone, and embedded in Epon 812 (4). Thin and thick sections were cut with glass knives in a Porter-Blum ultramicrotome. The thin sections, showing gold to silver interference colors, were contrasted with lead citrate (6) for electron microscopy. For anatomical observations under the light microscope, thick sections of 2-5 μ were stained with Azur II-Methylene blue (7). For control purposes, samples were collected from healthy plants at the same age and treated as described above.

## *Results*

Electron microscope examinations of thin sections of leaf tissues from Galego lime, Pera sweet orange, and Ruby Red grapefruit showed that in tristeza-infected materials, the most noticeable changes were at the vascular bundle, and that such differences were more pronounced in leaves infected with the severe strains.

In diseased plants, most sieve tubes and related cells at the periphery of the phloem bundle were crushed and probably dead (Fig. 1,A, see arrows); the same condition occurred occasionally at the central part of the bundle.

Within the phloem bundle, some modified sieve tube companion cells and parenchyma cells, characterized by a relatively electron-dense content, were observed (Fig. 1,A-D). The density of these cells was due to

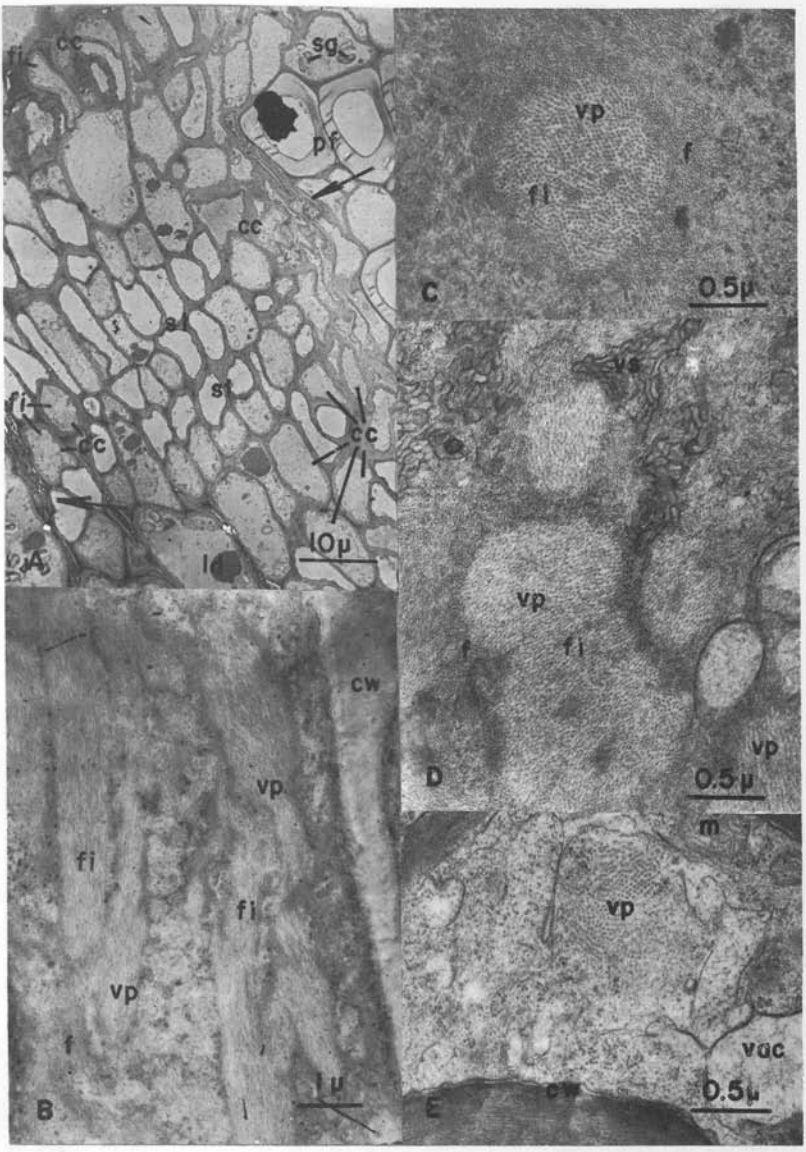


FIGURE 1. *Electron micrographs of thin sections from Galego lime leaves infected with a severe strain of tristeza virus (A-D) and with a mild type (E). A. Low magnification picture of a cross section of a phloem bundle. Arrows indicate crushed peripheral cells. B. High magnification of a longitudinal section of fibrous inclusions in chromatic cells, believed to consist of virus particles. C. and D. High*

a large number of fine fibrils, 6-8 mμ wide, arranged in parallel order in a compact mass and spaced about 15-20 mμ, center-to-center from each other. Embedded in this matrix were still identifiable cell structures, or their vesiculated remnants (Fig. 1,D), but usually no vacuole was present.

The most striking feature of these cells was the presence of 1 to 5 fibrous inclusions, variable in size (1-5 μ x 5-20 μ) and usually fusiform in shape. At higher magnifications, these inclusions appeared to be composed of elongated particles, about 10-12 mμ wide, and of unmeasurable length, disposed in a loose parallel array, and about 25-30 mμ center-to-center from each other (Fig. 1,B-D). In cross sections, they showed an axial hole, 3 mμ in diameter (Figs. 1,C-E), and they occasionally appeared to be arranged in a regular, crystalline pattern. Sometimes, signs of a helicoidal structure (Fig. 1,D) could be seen in these tubular particles. The particles of the fibrous inclusions appeared to be parallel to the fine fibrils of the surrounding matrix.

The frequency with which such abnormalities occurred in the phloem tissues was directly correlated with the severity of the virus strain studied and with the susceptibility of the host plant. The frequency decreased from Galego lime to Pera sweet orange to Ruby Red grapefruit. In some Galego limes infected with mild complexes and Pera sweet orange infected with the severe strain, apparently normal phloem cells occasionally contained aggregates of particles similar to those forming the fibrous inclusions (Fig. 1,E).

Cells with electron-dense cytoplasm, with or without fibrous inclusions, were restricted to the phloem of infected plants. Spongy or palisade parenchyma cells usually contained plastids in different stages of disorganization, and large starch grains. Some cells adjacent to and inside the vascular bundle contained large, dense droplets, probably lipid in nature. These changes in phloem tissues were never seen in leaves from non-inoculated control plants, although old leaves commonly showed plastid disorganization and starch accumulation as in diseased leaves.

In thick sections of diseased leaves, stained with Azur II-Methylene blue mixture and examined in the light microscope, the electron-dense

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*magnification view of cross and oblique sections, respectively. E. An apparently normal companion cell showing a small aggregate of presumed virus particles. Key: cc = electron-dense phloem cells, considered chromatic cells; cw = cell wall; f = fibril; fi = fibrous inclusions; ld = lipid droplet; m = mitochondrion; pf = phloem fibers; sg = starch grain; st = sieve tube; vac = vacuole; vp = virus particle; vs = vesicular remnants of cell structure.*

cells stained deep purple; the fibrous inclusions within them were weakly stained.

### *Discussion and Conclusions*

The tubular particles, forming the fibrous inclusions present in some modified phloem cells, appear to be identical to the tristeza virus particles found in leaf dip or partially purified preparations from infected citrus plants (3). They are always associated with the disease, and they have the same morphological characteristics, diameter, presence of axial channel, and the helicoid structure. The presence of these particles in phloem tissues only, supports pathological investigations which indicated that tristeza virus is a phloem-limited virus (1).

The morphological resemblance between tristeza virus particles and those of beet (*Beta vulgaris* L.) yellows viruses, *in vitro,* has been pointed out (3). Some aspects of tristeza virus inclusions were similar to those described for beet leaf tissues infected with the yellows virus (2), except for the banded form of inclusions, which were absent in the case of tristeza. Other differences are the lack of fibrilar matrix in beet cells bearing virus inclusions and the presence of virus in tissues other than the phloem in beet-yellows-infected beet leaves.

The electron-dense citrus cells containing the fibrilar matrix in which the aggregates of thread-like particles are embedded probably correspond to the chromatic cells described by Schneider (8) as the primary symptom of tristeza infection. Their location and internal characteristics of dense cytoplasm, few or no vacuoles or other cell structures, and fibrous inclusions, are the features that indicate this relationship. Furthermore, our electron microscope observations are in good agreement with Schneider's histochemical studies, suggesting the viral nature of the dark-staining masses present in chromatic cells.

The nature of the fibrils forming the matrix of the chromatic cells is not known. They may represent a virus precursor substance or products of cell degeneration. Similar material was never found in tissues from virus-free control plants.* The frequency with which virus inclusions occurred in infected plants correlated directly with the severity of the symptoms. This accords with the hypothesis that the severity of symptoms depends on the virus concentration.

*Fine particles with diameters similar to these fibrils were found (3) in partially purified preparations from both healthy and tristeza-infected Galego lime plants. However, their presence in preparations from healthy plants precludes a possible relationship with the fibrils present in chromatic cells.

Phloem tissues of Ruby Red grapefruit leaves were usually less affected than those of Galego lime or Pera sweet orange. In the case of seedling yellows virus in Eureka lemon [*C. limon* (L.) Burm. f.] and sour orange (*C. aurantium* L.), the changes were still milder (Kitajima, unpublished). These results agree with anatomical studies (8) which show that the major injury occurs in root tissues and that phloem tissues of stem and leaf are less affected.

Even in severely affected leaves, the phloem bundle is not completely collapsed. Although most of the peripheral sieve tubes and related cells are damaged, some apparently functional conducting tissues remain in the central parts. It is likely that this sheath formed by dead cells at the periphery of the phloem bundle constitutes a kind of barrier that impairs the passage of photosynthetic products toward the sieve tube. This would cause an accumulation of starch in the plastids of the leaves and would contribute to malnutrition of the roots, resulting in mineral deficiencies.

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NOTE.—After the manuscript was prepared for the Rome meeting of the IOCV, an article by W. C. Price appeared in Virology 29: 285-294, 1966, reporting electron microscope studies of tristeza virus in West Indian lime tissues.

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