Electron Microscopy of Tristeza-infected *Passiflora gracilis* Jacq.

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After Müller *et al.* (*9*) demonstrated that tristeza virus would infect plants of *Passiflora gracilis* Jacq., electron microscopic studies were carried out to demonstrate the presence of threadlike particles associated with tristeza virus in *P. gracilis*. Histology of leaf tissues was compared in both *P. gracilis* and Galego lime infected by tristeza virus. This paper describes some of the findings.

MATERIALS AND METHODS

Tests to detect threadlike, tristeza-associated particles (TAP) were made with negatively stained leaf-dip preparations (*6*) and thin sections of leaf tissues. For histological studies, small pieces of leaf tissues, including at least one veinlet, were fixed in 3 per cent glutaraldehyde and post-fixed in 1 per cent OsO_4 (both in phosphate buffer), dehydrated in acetone, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop IEM.

Samples were collected from *Passiflora gracilis*, both uninoculated and tristeza-infected, and from Galego lime infected with tristeza virus recovered from *P. gracilis*.

RESULTS

Leaf-dip preparations from tristeza-infected *Passiflora gracilis* consistently contained characteristic long, flexible threads, 10 nm × 1,000 to 2,000 nm, with periodic striations of 3 to 4 nm, and sometimes with an axial channel (fig. 1E). Such particles were never seen in preparations from uninoculated plants.

Ultrathin sections of leaf tissue from infected *Passiflora gracilis* plants revealed no major changes in epidermal or mesophyll cells, except for an unusual accumulation of starch grains in the plastids. In the vascular bundle, some cells adjacent to the sieve tubes had a mass of flexuous, elongated particles, about 9 nm thick, in their cytoplasm (fig. 1A, B, H). Similar particles have been described in many *Citrus* spp. infected with tristeza virus (*2, 7, 10, 11*) and are believed to represent the TAP seen in leaf-dip or purified preparations. These particles were usually arranged side by side, in a loose array. Cross-sections of the threadlike particles revealed a central, electron-transparent core (fig. 1F). Some of these cells also contained tubular P-protein (*3*) about 20 nm wide, which were easily distinguished from the threadlike particles by their larger diameter (fig. 1A, G). Another common feature of phloem cells containing flexuous threads was the presence of groups of vesicles, relatively uniform in diameter (0.1 to 0.2μm), with a meshwork of delicate fibrils (fig. 1A, B).

Similar threadlike particles and vesicles were also found in the phloem cells of the Galego lime infected with tristeza virus (fig. 1F, I). Intracellular threads from *Passiflora gracilis* and Galego lime were indistinguishable from those present in partially purified preparations of tristeza virus from Galego lime (fig. 1C).

Sieve tubes of both healthy and tris-

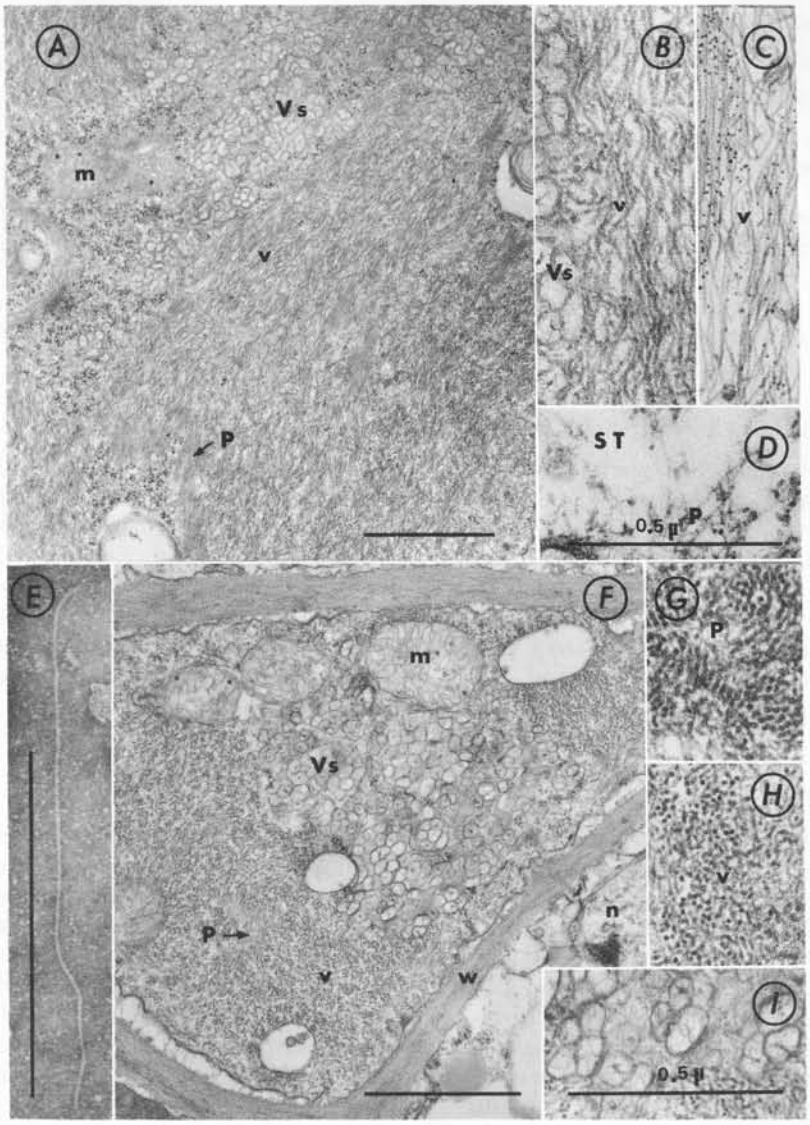

Fig. 1. Electron micrographs of tristeza-infected *Passiflora gracilis* Jacq. and Galego lime. (Unless otherwise stated, the bar in each micrograph equals 1 μm. *B* to *D* and *G* to *I* are in the same magnification.) *A*. Phloem cell of tristeza-infected *P. gracilis* leaf. Cytoplasm contains large number of threadlike particles (v) and vesicles (Vs). Bundles of thick, tubular P-protein (P) can be seen interspersed with threadlike particles; m=mitochondrion. *B*. Detail of *A*, showing threadlike particles (v) and some vesicles (Vs). Note meshwork of delicate fibrils in (Vs). *C*. Threadlike particles in pellets obtained by ultracentrifugation of partially purified preparation of tristeza-infected Galego lime. *D*. Fibrillar, extended form of P-protein (P) in the lumen of a sieve tube (ST) of uninfected *P. gracilis* leaf. *E*. Threadlike particle detected in a negatively stained leaf-dip preparation from tristeza-infected *P. gracilis*. *F*. Phloem cell of Galego lime leaf, infected with tristeza virus recovered from diseased *P. gracilis*. Threadlike particles (v) and vesicles (Vs) are also noticeable; m=mitochondrion; n=nucleus; P=protein; w=cell wall. *G*, *H*. Cross-section of tubular P-protein (P) and threadlike particles (v) present in phloem cells of tristeza-infected *P. gracilis*. *I*. Detail of *F* showing vesicles containing fine fibrils.

teza-infected *Passiflora gracilis* contained a fibrillar structure of about the same width as the threadlike particles seen in cells adjacent to the sieve tubes in tristeza-infected plants (fig. 1D). Fibrils present in uninfected plants probably represent the extended form of the P-protein, typical of mature sieve tubes (*3*), while those seen in tristeza-infected plants might be a mixture of such P-protein and TAP. Esau and Hoeffert (*5*) described a subtle difference between the extended form of P-protein and the beet yellows virion (BYV). This difference was noticed in isolated masses of either TAP (fig. 1B) or extended P-protein (fig. 1D), but was not distinguished within sieve tubes of tristeza-infected *P. gracilis* or Galego lime.

DISCUSSION

Electron microscopic studies of tristeza-infected *Passiflora gracilis* revealed that threadlike particles similar to those previously found in tristeza-affected citrus (*1, 2, 8, 10, 11*) were consistently found associated with symptoms following inoculation. This, together with the transmission and recovery data, indicates that *P. gracilis* is susceptible to tristeza virus and that TAP represent tristeza virus, rather than a by-product.

Similarity of threadlike particles found in the sections of pelleted tristeza virus preparations and those found within phloem cells adjacent to the sieve tubes is evidence that the threadlike particles seen *in vitro* are identical to the intracellular flexuous particles previously described (*2, 7, 10, 11*). They could not be the extended, fibrillar form of the P-protein because: (1) P-protein will not stand purification (Cronshaw, personal communication); (2) in its extended, fibrillar form, P-protein occurs only in mature sieve elements (*3*); and (3) there is a subtle morphological difference between intracellular TAP and the fibrillar P-protein.

The morphological similarity between TAP and BYV was considered evidence favoring the viral nature of TAP (*8*). Histological observations reported herein show some features common to both BYV-infected tissues (*4*) and the tristeza-affected *Passiflora gracilis* and Galego lime cells. Flexuous threads and groups of small vesicles containing fine fibrils are detectable in both. Such vesicles are believed to be related to the viral synthesis (*4*). Morphological similarity between BYV and the presumptive tristeza virus particles, as well as some biological properties, further favors the view that BYV and tristeza virus should be placed in a common group (*8*).

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