Hexagonal Tubular Structures in Sieve Tubes of Apple Leaves

M. Weintraub, R. Stace-Smith, and Bea Schroeder

Research Station, Agriculture Canada, 6660 N.W. Marine Drive, Vancouver, British Columbia, V6T 1X2.

ABSTRACT

Long, slightly curved tubular structures with a hexagonal cross-section and grouped into honeycomb arrangements were found in the sieve tubes of leaves from eight cultivars of apple trees. They were present in healthy control trees that had been indexed for, and found to be free from, known apple viruses, in trees infected with chlorotic leaf spot virus, and in trees that showed some witches'-broom symptoms of the foliage. The structures appear to be a normal component of apple leaf sieve tubes, and are not characteristic of apple trees suffering from proliferation disease.

Phytopathology 65:660-663

In the fall of 1973, a large group of Golden Delicious and McIntosh apples, on either standard or East Malling rootstocks, showed witches'-broom symptoms that were believed to result from environmental changes in water

availability, but might also have been symptomatic of apple proliferation disease (1). Since the presence of mycoplasmalike organisms has been demonstrated (9) to be associated with apple proliferation, and this finding has been amply confirmed (2, 11, 14), we undertook to examine the vascular tissues of leaves from a number of these trees in an effort to find the organisms.

METHODS AND RESULTS.—Samples were taken from the leaves of this group of Golden Delicious and McIntosh on 7 November 1973. The leaf tissues were prepared in the standard way, embedded in Epon 812, and the sections were stained with uranyl acetate and lead citrate. Thorough examination of the tissues failed to disclose the presence of mycoplasmalike bodies, although vesicles were found that might at first be taken for these organisms, until more detailed examination corrected this impression. This confirmed that the witches'-broom symptoms did not result from the apple proliferation disease, which as yet has not been found in Canada.

However, another peculiar structure was seen in profusion in the sieve tubes, often in large groupings (Fig. 1). In longitudinal section they appeared as tubules (Fig. 1, TS), while in cross-section they were packed into a honeycomb arrangement (Fig. 1, HS). The hexagonal cross-section of the individual tubules is seen in Figs. 1, 3 and 4. Measurements with a micro-comparator showed that the electron-dense wall was about 12 nm thick, and the center-to-center distance across two adjacent tubules was 31-32 nm. The hexagons were often slightly longer in one direction than in the other, possibly from oblique sectioning; the distance across the tubules in the longest dimension, from point-to-point in the hexagon was 34 nm. The elongated structure, slightly curved in longitudinal section (Fig. 2) demonstrates the difficulty in measuring the length of the tubule, but confirmed the diameter of the tubule as about 30-35 nm, and occasionally, up to 40 nm.

At the same time, we also examined leaf tissues from healthy control apple trees. These consisted of one tree each of the cultivars Early McIntosh, Gravenstein, and Scotia, all from a nursery at the University of British Columbia, which are indexed regularly for, and were free from, known virus diseases (sampled 23 July 1974); a Golden Delicious tree from the field; another Golden Delicious kept in a screen house; and a seedling of either McIntosh or Antonovka parentage, all from the Post-Entry Quarantine Station, Agriculture Canada, at Sidney, B.C., where the trees had been indexed for, and found free from, known apple viruses (sampled 28 August 1974).

The hexagonal tubules in a honeycomb arrangement were found within the sieve tubes of leaves from every tree examined, sometimes in the crevices between the thickened walls, where the lumen was reduced to a narrow space (Fig. 3), and sometimes apparently floating in the lumen (Fig. 4), although in the latter location, the free fragments may have broken off from larger groupings close to the cell walls.

Finally, we sampled on 17 September 1974 leaves and petioles from three apple seedlings (from Granny Smith, Dolgo, and Stahl's Prinz) infected with chlorotic leaf spot virus, from the experimental plots of W. R. Allen and T. R. Davidson at the Research Station, Agriculture Canada, Vineland Station, Ontario. Examination of the phloem tissues demonstrated the presence of the hexagonal tubular structures in the same honeycomb arrangement, identical in appearance with those that were first examined because of their witches'-broom symptoms, and with those in the healthy apple controls.

DISCUSSION.—Králík and Brčák (10) published a description and electron micrographs of apparently identical structures in the sieve tubes of sprouts from the roots of apple trees infected with proliferation disease. The only difference we can detect is their reported center-to-center dimension of 54 nm, as compared with our 30-35 nm. They did not find these structures in healthy controls. Although they concluded that "their (i.e., the tubule's) connection with mycoplasmas could not be proved . . . . ", they also concluded that these structures were distinct from any normal plant cell organelle. Because Králík and Brčák did not find the tubules in healthy controls, we are concerned that their discovery of these structures in infected trees may lead others to the conclusion that the presence of hexagonal tubules indicates infection with the apple proliferation disease.

Like Králík and Brčák, we also do not know the nature of the tubular structures, although we hope that experiments underway will demonstrate their chemical composition. Since they are found in sieve tubes, they invite comparison with P-protein. Generally, P-protein in differentiating sieve elements is seen in the form of tubules, variously reported as 17-24 nm in diameter, with an electron-lucent core of about 5 nm and wall thickness of 7-8.5 nm (4, 6, 13), and this appearance is characteristic even of nuclear P-protein in sieve elements (7). These tubular elements may form large aggregates, some of which are almost crystalline (4, 8, 13), and in soybean they form what appears to be true crystals (15). The next stage in mature sieve elements within the vascular system (5) normally involves dispersal of these bodies of tubules into fibrils 6-7 nm (13) and up to 14.9 nm (3) in diameter and tubules about 10 nm in diameter (13).

It is with the larger tubules in aggregated form that the hexagonal tubules of apple leaves may be compared. When this is done, obvious differences emerge: the diameter of the hexagonal tubules in apple is 30-35 nm, up to 40 nm, and according to Králík and Brčák (10) may even be up to 54 nm, while the generally accepted diameter for the larger P-protein tubules is 17-23 nm (4, 12, 13); wherever P-protein aggregates into paracrystals or crystals, there is always an electron-lucent space separating the tubules, even though there may be fine interconnecting strands between the tubules (3, 12, 13), and the arrangement of the tubules within the aggregates may occasionally be in hexagonal configurations formed by groupings of six tubules (12, 13), whereas the tubules in apple leaves are themselves hexagonal in shape, and form a tight honeycomb arrangement with no space between individual tubules; and finally, P-protein is frequently found in phloem parenchyma and in companion cells (4), whereas the hexagonal tubules in apple are restricted to sieve tubes. Because of these differences we are not inclined to identify the hexagonal tubules in apple sieve tubes as P-protein, although it is not impossible that in apple, P-protein assumes this peculiar configuration.

CONCLUSIONS.—We emphasize that these

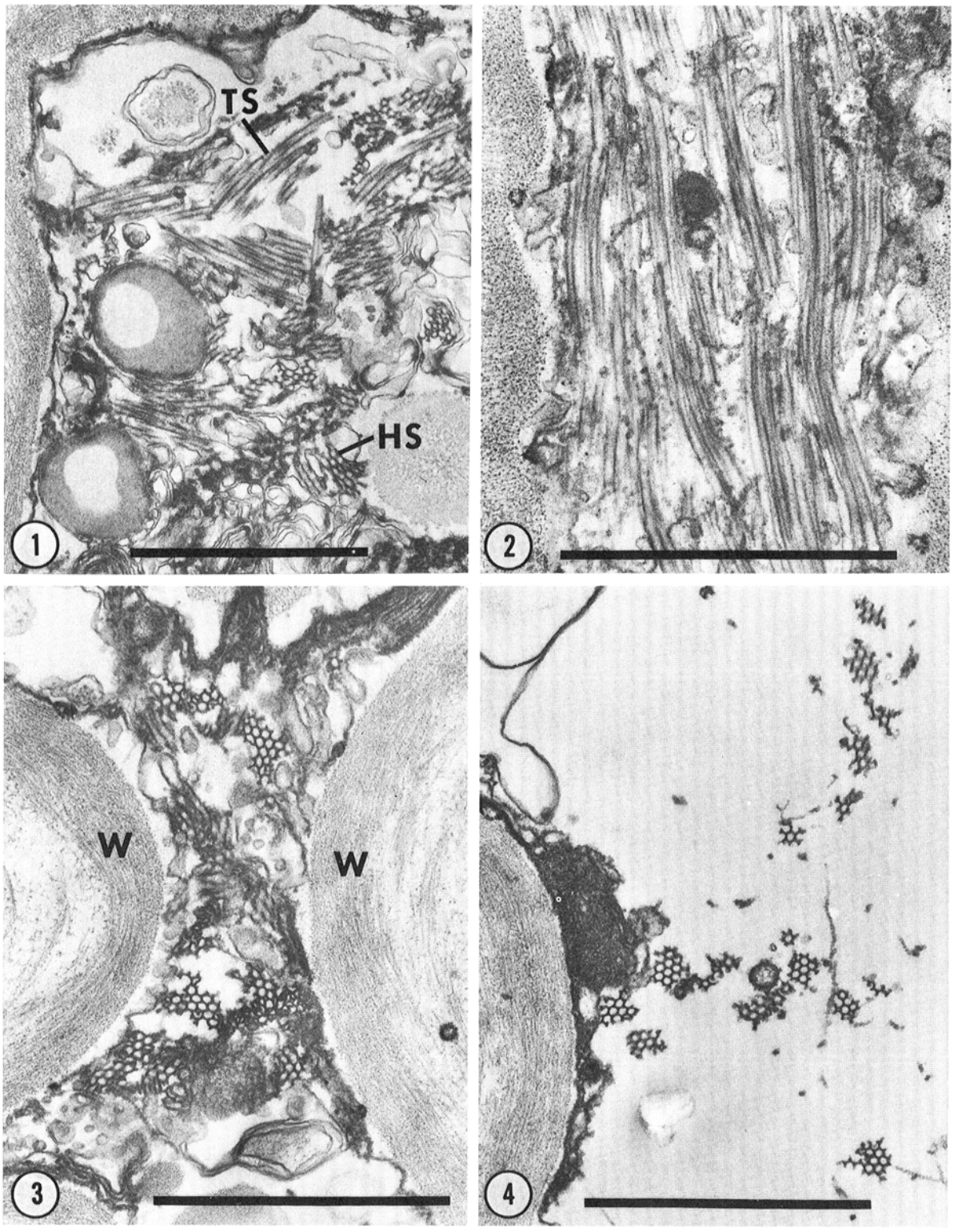

Fig. 1-4. Electron micrographs of apple leaf sieve tubes. **1)** Tubular structures in longitudinal section (TS) and in honeycomb aggregates in cross-section (HS). Cultivar Golden Delicious with witches'-broom symptoms (× 39,000). **2)** Higher magnification of the tubular structures in longitudinal section, showing somewhat curved shape. Cultivar Golden Delicious with witches'-broom symptoms (× 55,400). **3)** Hexagonal tubules in crevice of sieve tube between the thickened cell walls (W). Cultivar Gravenstein, healthy control (×48,700). **4)** Hexagonal tubules, some attached to cell contents, others free in sieve tube lumen. Cultivar Gravenstein, healthy control (×47,000). Scale bars represent 1.0 μm.

structures were found in all eight cultivars and seedlings so far examined, including healthy trees, chlorotic leaf spot virus-infected trees, and trees with and without symptoms of proliferation. The evidence therefore indicates that the tubular hexagonal structures in honeycomb arrangements are a normal and ubiquitous component of the sieve tubes of apple leaves.

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