Electron Microscopic Investigations on Volkamer Lemon Affected by Impietratura

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Impietratura disease has been known for about a century in the Mediterranean area. The diagnostic symptoms observed on fruit are summer drop, reduction in size, various patterns of skin hardening (with or without protuberances) and albedo gumming. The vascular bundles of the peel and central core, and the young xylem of pedicels, twigs and branches show necrosis and gum pockets. Since the disease is graft-transmissible, the causal agent is assumed to be a virus, but many attempts to isolate or to visualize it in the affected tissues have failed (Catara *et al.*, 1977). This paper reports 2 years' results of electron microscopy undertaken to find the causal agent in affected cells, and to investigate the histological and cytological effects of the disease.

MATERIALS AND METHODS

Inoculated seedlings of Volkamer lemon with a high percentage of diseased fruits each year were used. In spring, fertilized and unfertilized ovaries, floral pedicels and fruit in the initial stage of development (0.5-1.5 cm diam) were collected from stems which had yielded affected fruit previously. In summer, leaves, petioles, fruit, and twig and peduncle bark were taken from twigs bearing fruit with symptoms.

Samples were cut into 1 x 2-mm pieces, fixed in buffered 3 per cent glutaraldehyde, postfixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite. After fixation, the outer layers of bark were removed to allow a better penetration of the resin. Some ovaries were subjected to high-speed centrifugation (66,000 *g* x 1 hr) before fixation to facilitate the detection of foreign particles.

Sections, 1 to 5μ thick, were stained with methylene blue and basic fuchsin, and examined with a Leitz Orthoplan light microscope.

Ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Siemens Elmiskop 1A.

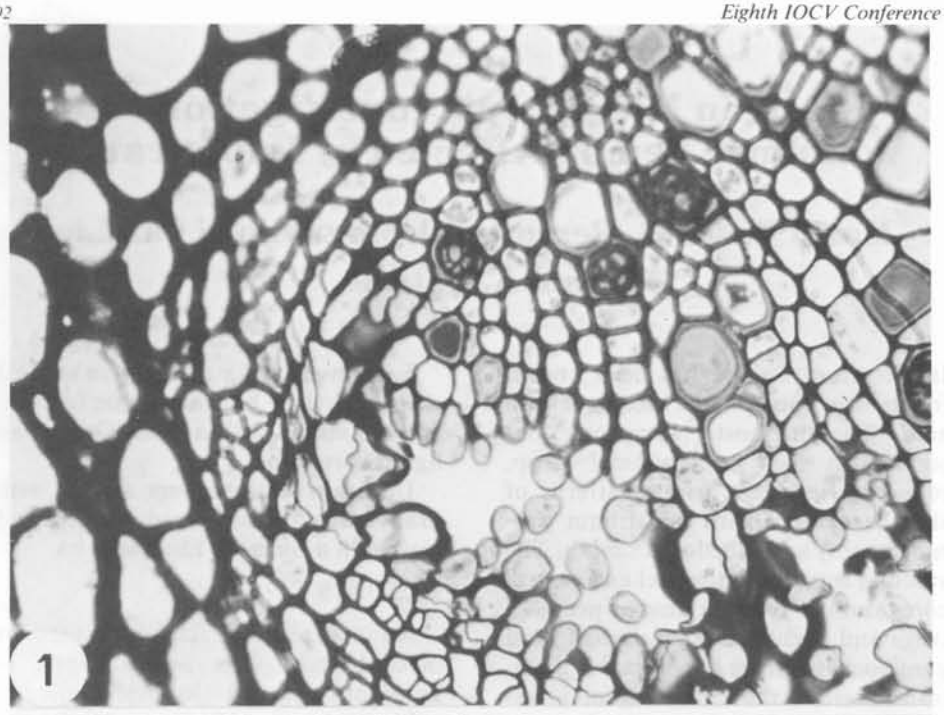
RESULTS

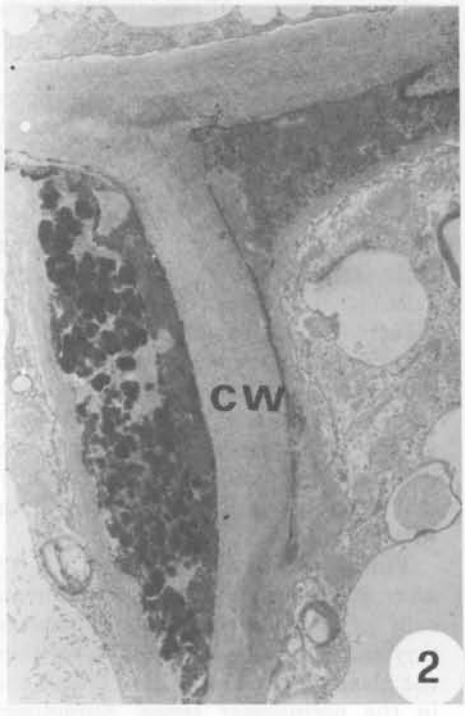
No organisms, such as viruses or mycoplasmas, were seen in spite of numerous observations on the phloem, xylem, cortex, albedo, parenchyma, and chlorenchyma. High-speed centrifugation of samples before fixation also failed to reveal anomalous particles. Nevertheless, some ultrastructural alterations were observed in the organs examined.

Ovary. The only anomalous features observed were in xylem parenchyma cells of the carpellary bundles. In these cells, the plasmalemma was more electron-opaque than normal and highly invaginated into the cytoplasm. Ribosomelike particles attached to these invaginations were sometimes visible. Short rows of highly electron-dense particles and an unusual number of long polysomes were also visible in the cytoplasm.

Floral peduncle. Before and shortly after anthesis, the xylem parenchyma cells of the vascular bundles showed tiny electron-opaque droplets between the plasmalemma and the cell wall and in the perinuclear space. Sometimes larger electron-opaque droplets were visible within the cell wall and along the middle lamella and/or a large mass of electron-dense substance interposed between adjacent cell walls (fig. 2).

Bark and young leaves. No alteration was found in the bark. In young leaves,





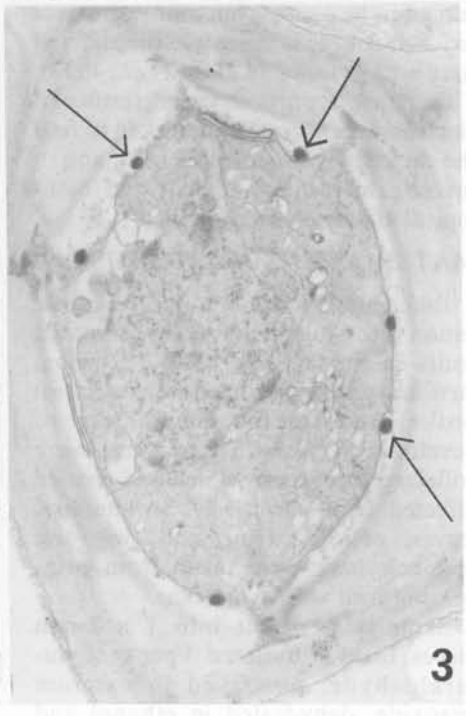


Fig. 1. Schizolysigenous cavity in an axial bundle of a fruit of Volkamer lemon affected by impietratura. Vessels filled with gumlike substances are visible.

Fig. 2. Floral peduncle of Volkamer lemon affected by impietratura. Electron-opaque granular substance in the intercellular spaces of adjacent xylem parenchyma cells of a vascular bundle; cw = cell wall.

Fig. 3. Leaf petiole of Volkamer lemon affected by impietratura. Lipophanerosis of the plasmalemma in a differentiating tracheid of a vascular bundle (arrows).

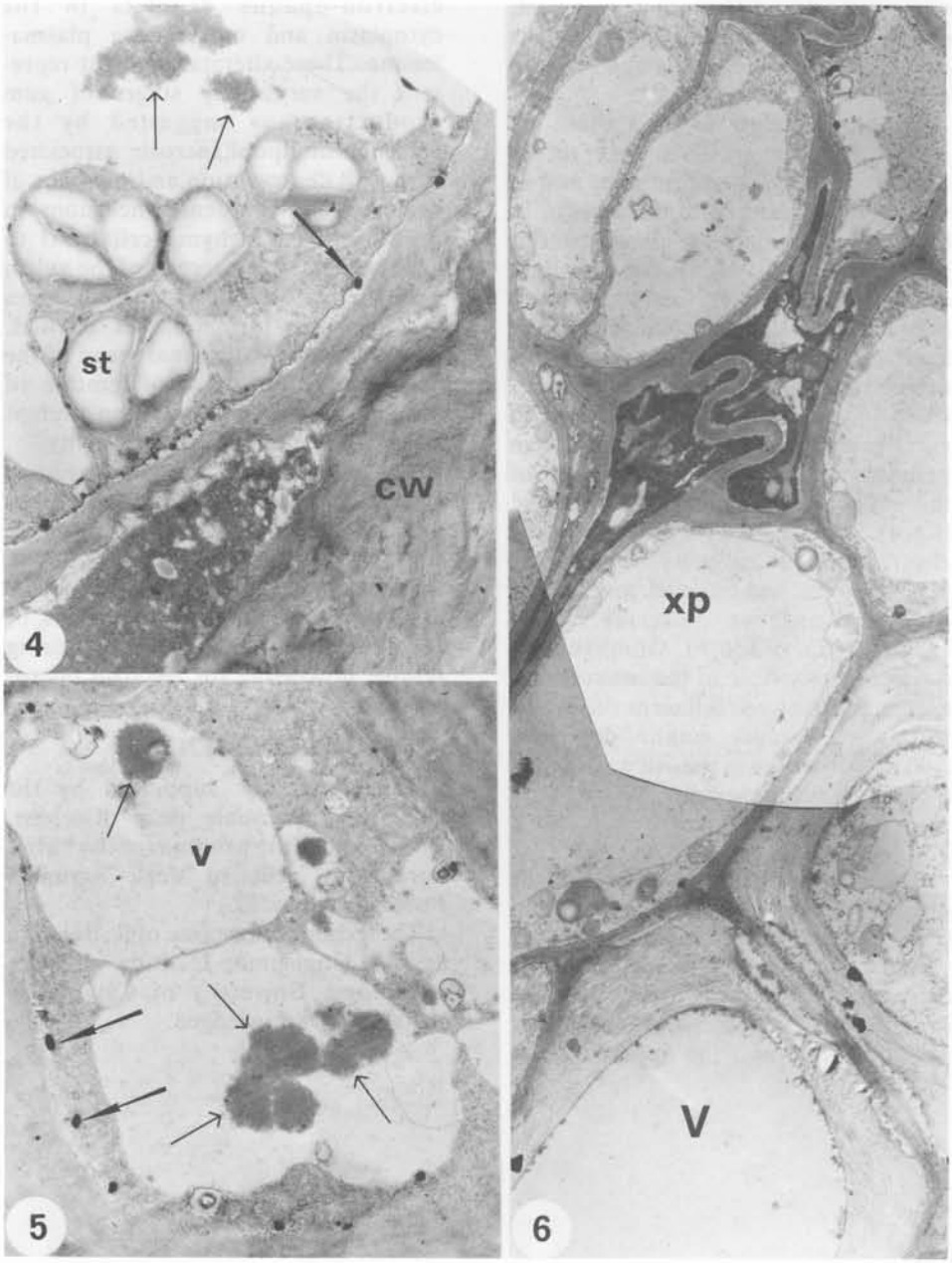


Fig. 4 and 5. Parenchyma cells of the albedo of affected Volkamer lemon fruit showing lipophanerosis in the plasmalemma (large arrows) and electron opaque vacuolar inclusions (small arrows); cw = cell wall; v = vacuole; st = starch.

Fig. 6. Axial bundle of an affected Volkamer lemon fruit. Necrosis of a group of xylem parenchyma cells and proliferation of an adjacent cell which remains functional (top); V = vessel; xp = xylem parenchyma.

some of the differentiating vessels showed tiny electron-opaque droplets between the plasmalemma and cell wall (fig. 3).

Developing fruit. In fruit about 1.5 cm in diam, alterations were recognizable in the vascular bundles and in the parenchyma cells of the albedo. In the vascular bundles, schizolysigenous gum pockets, also visible by light microscopy (fig. 1), were observed between phloem and xylem. They were lined and/or filled by a cicatricial tissue originating from adventitious meristems. Some vessels were filled with gumlike substances. Necrosis of xylem parenchyma cells and proliferation of the adjacent cells were also observed (fig. 4). Some cells in the albedo showed electron-opaque droplets between the plasmalemma and cell wall, and masses of electron-dense material in the vacuoles (fig. 5 and 6). Gumlike substances were visible in the intercellular spaces and between adjacent cell walls. In advanced stages, gummy degeneration was observed in the cell walls of the albedo parenchyma cells.

DISCUSSION

The earliest cytopathic effects of the disease were observed in the ovary and the floral pedicel, and suggest increased secretory activity, as indicated by the increased number of polysomes, the invaginations and convolutions of the plasmalemma, and the appearance of electron-opaque droplets in the cytoplasm and outside the plasmalemma. These alterations might represent the very early stages of gum production, as suggested by the presence of lipophanerosis associated with wall degeneration and presence of vacuolar electron-dense inclusions in the albedo parenchyma cells next to gum pockets. The necrosis of the xylem parenchyma cells of vascular bundles precedes the formation of cavities, which represents the final stage of the degenerative process. Proliferation of the adjacent cells is probably an attempt to limit and circumscribe the cavity.

The failure to find foreign organisms, even in the tissues which transmit the disease when grafted on a healthy plant, suggests that the infectious agent is too small or too irregularly distributed to be easily revealed in the affected tissues, or so labile that it disintegrates during sample processing for electron microscopy.

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