The Ultrastructure of a Rickettsialike Organism from a Peach Tree Affected with Phony Disease

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ABSTRACT

Electron microscopy revealed a rickettsialike organism in vessels of roots of a peach tree affected with phony disease. None was found in similar roots from four nearby healthy trees. The most prominent feature of the cells of the organism is the circumferential folds of the outer wall which are oriented in a regular annular or spiral pattern. In thin sections the outer wall appears notched, wavy, or rippled. The cells are about 2.3 $\mu \times 0.35$ μ, slightly smaller than a similar organism associated with Pierce's disease in grape. A double-layered outer wall and a double-layered cytoplasmic membrane surround the cell contents which appear similar to those illustrated for rickettsiae. A matrix seems to surround some groups of cells. Strands that appear made up of subunits protrude from the outer wall or lie free among the cells. Also among the cells are particles about 28-nm diam that occur singly, in short chains, or clusters. These may be broken portions or subunits of the strands.

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Disease transmission by grafting led to the belief (4) that a virus is the etiological agent of phony disease of peach. Subsequent work (10) showed that the disease was spread by leafhopper vectors, which was interpreted as added proof of its viral nature. No entity, however, has heretofore been associated with phony disease. Recently we found rickettsialike organisms in grapevines affected with Pierce's disease (1), and workers in Florida (2) confirmed this finding. Pierce's disease of grapevines and phony peach have common attributes. The etiologic agents of both are confined to xylem tissues of their hosts (3, 5) and have some common insect vectors (7). This prompted us to examine leaf and root tissues from a peach tree affected with phony disease with the electron microscope. This paper is a report on the ultrastructure of an organism found by such examination.

MATERIALS AND METHODS.—Leaves from a peach tree affected with phony disease were collected along with leaves from four healthy control trees at the Southeastern Fruit and Tree Nut Research Station, Byron, Georgia, and forwarded to Davis, California on 12 July 1972. The tissues were killed and fixed and sections were prepared for electron microscopy as described previously for grape leaf tissue (1).

A second collection of materials was made from the same trees on 20 September 1972, and forwarded to Davis. Included were leaves and roots with small rootlets about 2-mm or less in diam. Tissues from both the leaves and rootlets were prepared for electron microscopy.

RESULTS.—No organisms were found in tissue from peach leaves in either collection, nor were any found in leaves from healthy controls. This was not unexpected because Hutchins et al. (6) reported that phony disease was transmitted only occasionally with tissue taken from affected peach trees above ground level.

A rickettsial organism was found in the lumen of each of over 1,000 sections of the vessels of roots from the diseased tree but not in any of 1,200 sections of the vessels of roots from the four nearby trees that showed no symptoms of phony disease. The organism occurred irregularly in several adjacent vessels and was usually more abundant in one of the vessels than in the others. The cells of the organism typically are elongate with rounded or tapered ends. Minor variations in

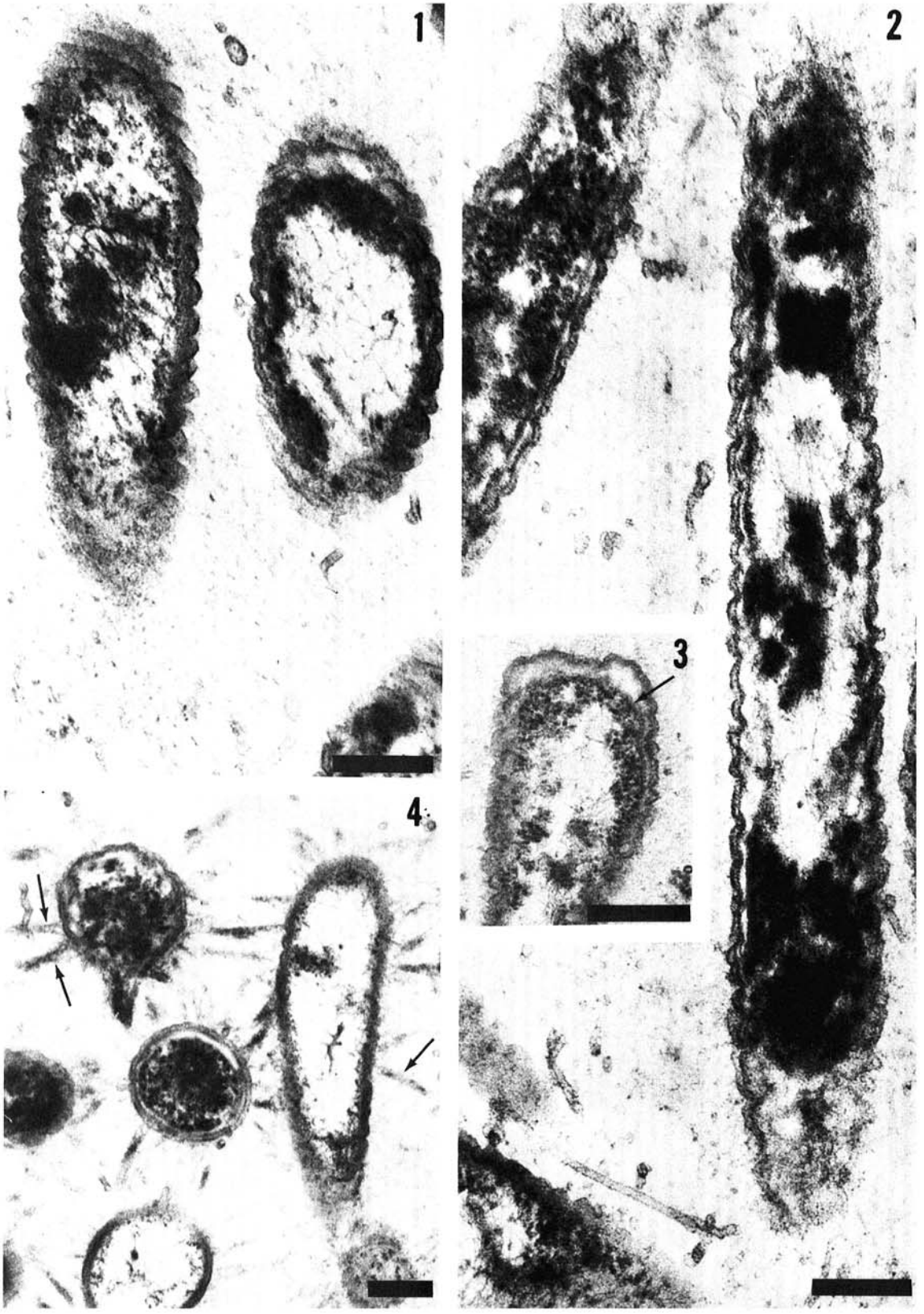

Fig. 1-4. Ultrastructure of rickettsial cells in cross sections of vessels from roots of phony-affected peach trees. **1)** Rippled cell wall showing spiral arrangement of folds or ridges near the ends of the cells. **2)** Two osmiophilic layers of the cell wall with one electron lucent space between layers and another adjacent to inner face of the wall. Note smooth, nonrippled cytoplasmic membrane, electron-dense strands are scattered among the cells and in some cases appear attached to the outer walls. The strands are made up of subunits. **3)** Portion of a longitudinal section showing double-layered cytoplasmic membrane (arrow). **4)** Broad osmiophilic bands occasionally seen among groups of cells. Particles and unitized strands seem associated with the outer wall (arrows). Scale bars = 0.2 μ.

morphology were noted. The approximate maximal length of the cells was 2.3 μ and the width was about 0.35 μ. The size of this organism is slightly smaller than a similar one found in grape.

The ultrastructure of the organism associated with phony peach is similar to other described rickettsiae (8). The outer cell wall of the organism from peach is strikingly rippled. In oblique longitudinal sections (Fig. 1), the ridges or folds, which account for the rippled appearance of the outer walls, tend to overlap especially near the ends of the cells and appear to coil or wrap around the cells spirally or circumferentially throughout their length. The average width of each ridge in section is about 62 nm, but there is considerable variation even in the same cell. The height of each projection or depth of a notch where these are apparent is 40-50 nm. These measurements are approximately the same as those for *Rickettsia prowazeki,* the typhus pathogen (9). With *R. prowazeki,* no special orientation of the folds of the outer wall was reported.

In longitudinal sections, four electron-dense layers are seen, two outer and two inner, surrounding the cells. The two outer layers, both of which are rippled (Fig. 2), comprise the cell wall and the two inner layers, the cytoplasmic membrane (Fig. 3). The outer layers are separated by an electron-lucent space about 10- to 15-nm wide and a similar space occurs between the cell wall and the cytoplasmic membrane. Each layer of the cell wall is 6 nm wide and the double-layered cytoplasmic membrane is slightly wider. The two layers of the wall are more or less parallel and appear notched or serrated, but the cytoplasmic membrane, where it can be seen clearly, is smooth.

Some cells possess very fine to broad osmiophilic lines

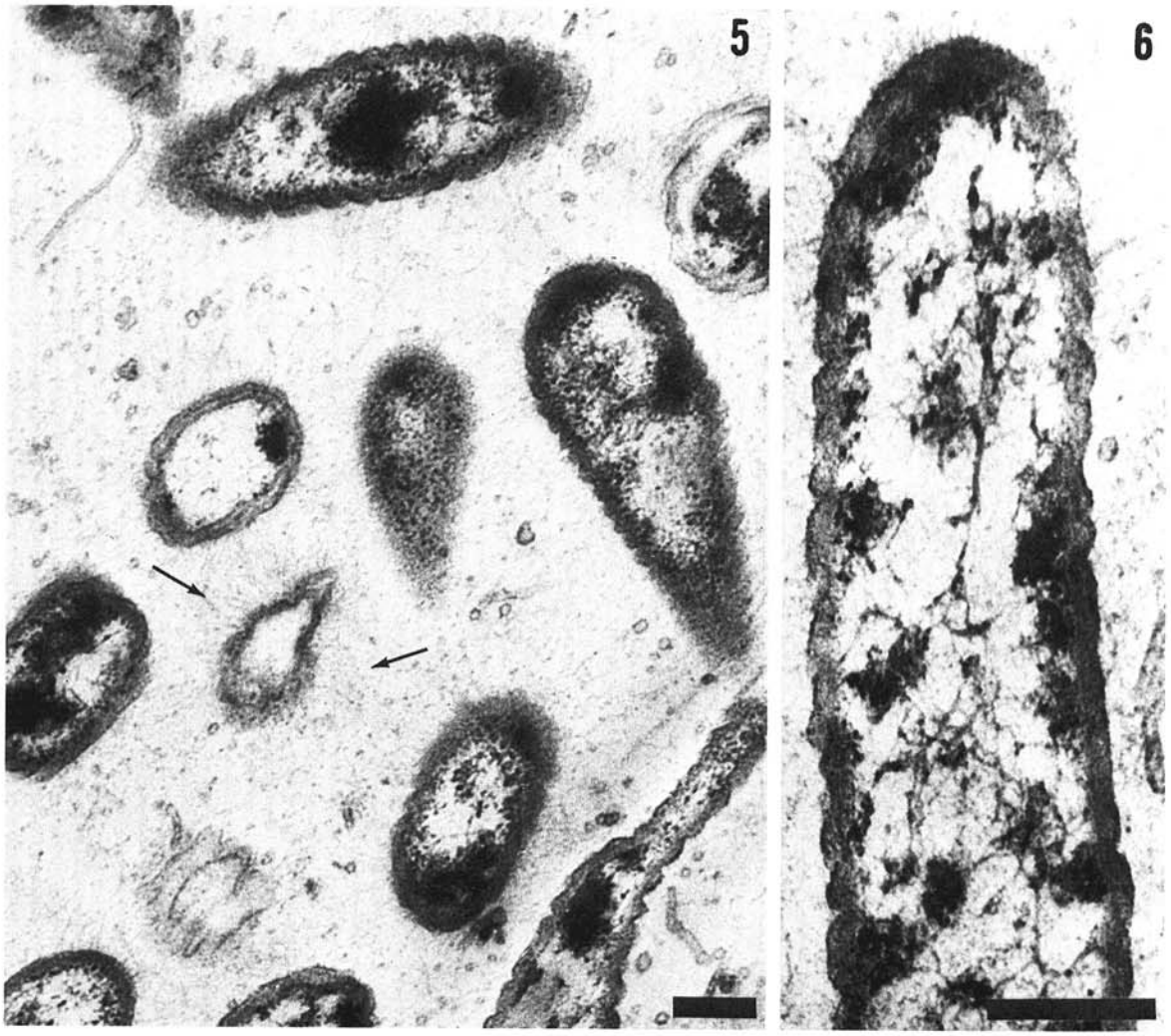

Fig. 5, 6. 5) Fine lines radiating from cells and similar appearing electron-dense ground substances among the cells may be matrix (arrows). Ribosome-like granules are concentrated near the periphery of the cells. Note unitized strands and particles also identified in Fig. 2 and 4, and a portion of the outer wall of a cell showing annular arrangement of ridges or folds (lower left). **6)** Near median longitudinal section of a portion of a cell showing abundant DNA-like network. Scale bars = 0.2 μ.

that radiate from the periphery of the cell wall (Fig. 4, 5). These may represent a sheath that has been modified by preparative procedures. Some cells have strands protruding from the wall and similar strands are scattered about in the lumen of the vessels among the rickettsial cells (Fig. 2, 4, 5). Close observation reveals transverse lines in the strands, suggesting that they are made up of subunits. Some particles that lie free in the lumen of the cells are angular, about 28 nm in diam, and occur singly, in short chains and in clusters, and may be broken strands or subunits of the strands. The strands and subunits may be comparable to "vesicles" described for the "Z" strain of *R. prowazeki* (9) and may originate in some manner from the cell wall; or, they may represent completely separate entities.

In general, the contents of the cells are similar to those described for other rickettsiae (8). The cytoplasm of the cells contains osmiophilic granules, probably ribosomes (Fig. 5), about 15-nm in diam; fine threadlike strands, probably DNA (Fig. 6), that often form a network; and masses of electron-dense material. The ribosome-like granules usually are most abundant around the periphery of the cell in median as well as in peripheral sections (Fig. 5).

The rickettsialike organism found in a peach tree affected with phony disease resembles the one described from grape, but several differences are noted. The rippled walls are more pronounced, the unitized strands and subunits are not noted in grape, and the organism from peach is slightly smaller than the one from grape. In both organisms, the cell walls are folded or ridged and the prominent ridges in the peach organism are oriented in a regular pattern.

The organisms found associated with Pierce's disease and phony peach are both larger than a rickettsialike organism that Windsor & Black (11) found associated with clover club-leaf.

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