Occurrence of a Rickettsia-like Bacterium in the Xylem of Peach Trees with Phony Disease

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ABSTRACT

By electron microscopy, a pleomorphic bacterium was observed in constant association with the xylem of roots and leaves from peach trees having phony disease symptoms, but not in the xylem of healthy trees. The bacterium did not grow on any of four general-purpose bacterial media. The causal agent of phony disease of peach may be a rickettsia-like bacterium.

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Phony disease of peach is a serious limiting factor in the production of peaches in the Southeastern United States. Phony disease symptoms include dark-green, flattened leaves, dwarfed new growth, and a general stunting characterized by shortened internodes (4). The causal agent is transmissible by grafting (4), and by several sharpshooter vectors of the Tettigellinae (7). Since no fungal or bacterial organism could be associated with the disease, the causal agent has long been assumed to be a virus.

A rickettsia-like organism was recently found to be closely associated with Pierce's disease of grape and may be the causal agent (2, 3). Phony disease of peach and Pierce's disease of grape have similar symptoms in the xylem of the hosts (1). The causal agent of each disease is transmitted by members of the same leafhopper subfamily and appears to be closely associated with the xylem of the host (5). These similarities between phony disease and Pierce's disease prompted this examination by electron microscopy of vascular tissue of diseased peach trees.

'Maygold' and 'Junegold' peach trees naturally infected with phony disease were used in this study. Diseased trees had typical symptoms, and a positive reaction was obtained with the chemical test for phony disease (4). Trees chosen as healthy were from orchards free of phony disease symptoms and a negative reaction to the chemical test occurred. Samples of tissue, 0.5- to 1.0-mm long, were taken from small roots (<5-mm diam) and midveins of leaves. The tissue samples were fixed overnight at 4 C in 2% glutaraldehyde-2% paraformaldehyde in 0.05 M collidine buffer, pH 7.3, followed by two 10-min rinses in 0.1 M phosphate buffer. The samples were left in 0.1 M phosphate buffer for approximately 2 days while being mailed from Leesburg, Florida to College Station, Texas. The samples were postfixed in 1% OsO_4 overnight at 4 C. They were washed in distilled water, dehydrated in an alcohol and acetone series, and embedded in a Spurr epoxy-resin mixture (6). Thin-sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Philips EM-300 electron microscope.

A pleomorphic bacterium, similar to the rickettsia-like bacterium described in grapevines with Pierce's disease (2, 3), was found in the tracheary elements of small roots and leaf midveins of peach trees with phony disease (Fig. 1). The bacterium was found in every thin section from diseased trees. The distribution of bacteria illustrated in Fig. 1 was common in both leaf and root, but was not characteristic of all xylem elements in any single section across a root or leaf vein. In each transverse section, many elements of the xylem were clear of bacteria. In the roots, the organism seemed to be more common in the small thick-walled tracheids than in the larger vessels. The organism was not seen in the xylem parenchyma or in the phloem. The bacterium was found in eight diseased trees and was not found in sections from five healthy trees.

The rickettsia-like bacteria were most commonly 0.25-0.4 μm by 1.0-3.0 μm rod-shaped bodies with well-defined cell walls about 200-300 Å thick (Fig. 1, 2). The walls were usually ridged in a periodic manner and the ridges appeared to spiral around the long axis of the organism. The electron density of the bodies, the thickness and appearance of the walls, and the amount of ripple in the walls were variable, perhaps reflecting different stages of development or deterioration of the cells (Fig. 1, 2). A finely granular staining substance was usually present in the space between the bodies (Fig. 1, 2).

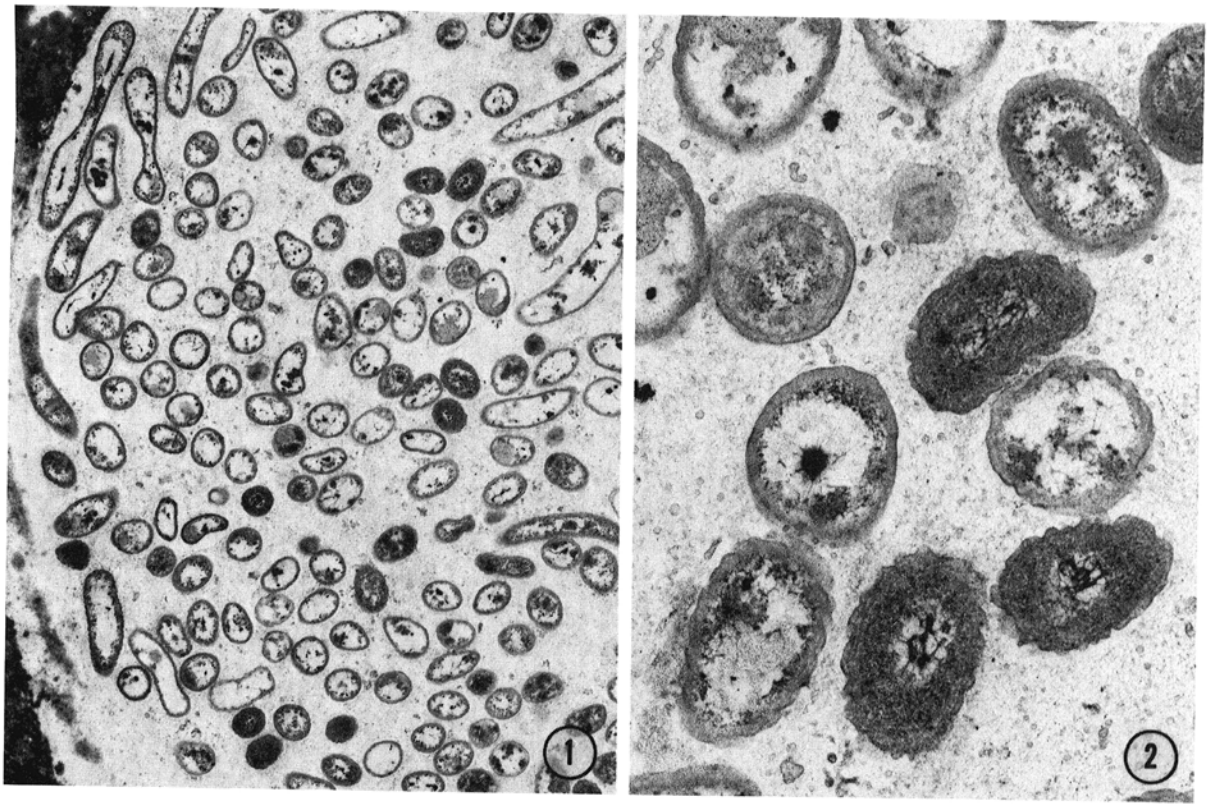

Fig. 1-2. Micrographs of rickettsia-like bacteria from peach trees with phony disease. The bacteria are generally rod-shaped with well-defined cell walls about 200-300 Å thick. The walls are usually rippled (or ridged). A finely granular staining substance is usually present in the space between bacteria. **(Fig. 1** × 14,000; **Fig. 2** × 60,000).

Attempts were made to isolate the bacterium from sections of roots and midveins contiguous to those examined with the electron microscope. The roots and midveins were surface-sterilized and rinsed in sterile water. Sections (12-mm) of the roots or midveins were excised and triturated in 2 ml of buffered saline. These suspensions were streaked onto nutrient agar + glucose, King's medium B, yeast extract-glucose-calcium carbonate agar, and skim milk agar. These inoculated plates were observed for 14 days. The organism present in the xylem of trees with phony disease did not grow on any of these four general-purpose media.

In morphology, tissue localization, and failure to grow readily in cell-free media, the bodies associated with phony disease are similar to the rickettsia-like bacterium occurring in grape with Pierce's disease. These results indicate that phony disease may be caused by a rickettsia-like bacterium rather than by a virus. Proof that the rickettsia-like bacterium associated with phony disease is the causal agent, awaits the completion of Koch's postulates.

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