A Method for Observing Rickettsialike Bacteria Associated with Phony Peach Disease

W. J. French

Assistant Professor of Plant Pathology, University of Florida, IFAS Agricultural Research Center, Monticello 32344.

Florida Agricultural Experiment Station Journal Series Paper No. 4986.

ABSTRACT

Rod-shaped bacteria were observed with phase contrast microscopy when root and stem sections from peach trees affected with phony disease were treated with 0.1 M KOH. The bacteria were similar in size, shape, and host-tissue localization to the rickettsia-like bacteria which were observed by electron microscopy in tissues from the same trees. The bacteria could not be cultured on artificial media. No bacteria were observed in tissues from healthy trees.

Phytopathology 64:260-261

Additional key words: Prunus persica.

Rickettsialike organisms were observed in peach tissues affected with phony peach disease when ultrathin sections of roots were examined with an electron microscope (5). The rickettsialike bacterium associated with phony disease are similar in morphology and tissue localization to the organisms occurring in grapes with Pierce's disease (3, 4). Hopkins and Mortensen (6) reported a remission in Pierce's disease symptoms in grape treated with tetracycline. Similar antibiotic treatments have been initiated on trees affected with phony. In the course of selecting trees for treatment and in evaluating the results, the need arose for a rapid method of examining tissues for the presence of rickettsialike organisms. Since the organisms associated with phony peach are within the size limitations of the light microscope, 0.25-0.4 μ in diam and up to 3.0 μ long (5), diseased material was prepared for examination by light microscopy.

Root and stem sections were selected from eight peach trees which had typical phony symptoms and in which the presence of rickettsialike bacteria was previously determined by electron microscopy.

Healthy roots were selected from asymptomatic trees in which the organisms were absent when examined with an electron microscope. Tissue samples 5 mm in diam and 5 mm long were cut from root and stems which were free of visible wounds, decay, and discolorations. Fresh samples were cut into transverse sections 10 to 15 μ thick with a freezing microtome (American Optical Model 888) and mounted in a drop of sterile water on a microscope slide. All microscopy was with a Leitz Ortholux, equipped with a Zernike-type phase contrast system.

When diseased tissues were examined with the phase contrast system, rod-shaped bacteria from 1-3 μ long were found in xylem vessels of roots and stems. The rods, usually few in number, were located close to the cell wall within the lumen. In oblique sections the cell wall tended to obscure those rods located along the periphery of the lumen.

Transverse sections of roots from phony peach had variable numbers and sizes of gummed areas in the xylem. Gum occurred in vessels, tracheids, xylem parenchyma, and ray cells. A similar pattern of gumming in phony peach roots was observed by Esau (2). Gum partially or completely filled the vessel and obscured the lumen. The gum often contained refractive granular material which could not readily be distinguished from aggregates of bacterial rods.

Many of the common bacteriological stains and modifications of Castaneda's (1) and Machiavello's (9) methods for staining Rickettsias were tested to differentially stain the rods in fresh sections; none was effective.

When sections were gently boiled in a few drops of water on a microscope slide, the rods were released into the mounting water and were readily observable. It was also found that an (unheated) aqueous solution of KOH or NaOH produced the same results with remarkable speed. NaOH quickly caused deterioration of the sections and the bacterial rods, whereas KOH was less caustic. Concns of KOH ranging from saturation to as low as .01 M were effective in releasing large numbers of rods from infected tissues. However, the higher the concn of KOH used the greater the mass of cellular debris released into the medium, and the more difficult the observation became. The optimum concn of KOH was found to be 0.1 M but occasionally 1.0 M and 10.0 M were useful with some tissues.

Tissues were taken from roots and stems of healthy trees and trees affected with phony disease for attempted culturing. Sections were surface-sterilized in a sodium hypochlorite solution (0.5%), plated and incubated on nutrient agar, King's medium B (7), and yeast extract-glucose-calcium carbonate agar (8). The organism present in the xylem of trees infected with phony peach disease did not grow on any of the artificial media used.

In summation, bacteria were observed when KOH was applied to sections of roots and stems from all eight trees which had symptoms of phony disease and which had rickettsialike organisms in xylem vessels when observed with an electron microscope. No bacteria were observed in roots or stems of the five trees which were clinically healthy. The reliability of the KOH method of detecting bacteria in phony diseased xylem tissue was tested further on numerous phony and healthy trees and gave consistent, reproducible results. The method which I have adopted for routine use is as follows:

1. Select unblemished roots or branches 2-7 mm in diam and 5 mm long or cut larger pieces to these dimensions.
2. Cut sample into transverse section 10-15 μ thick using a freezing microtome. Place sections in watch glass containing water for few min to remove excess starch granules.
3. Clean blade thoroughly before sectioning next sample.
4. Mount sections in drop of water on slide and cover; observe with X400 phase contrast system.
5. Add 1-2 drops 0.1 M KOH to edge of cover glass and observe the outer periphery of sections for release of bacteria into the surrounding liquid. Scan vessel lumina for presence of bacteria.

LITERATURE CITED

1. CASTANEDA, M. R. 1930. A new stain for Rickettsia bodies. J. Infect. Dis. 47:416-417.
2. ESAU, K. 1948. Anatomic effects of the viruses of Pierce's disease and phony peach. Hilgardia 18:423-482.
3. GOHEEN, A. C., G. NYLAND, and S. K. LOWE. 1973. Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341-345.
4. HOPKINS, D. L. 1973. Rickettsia-like bacterium associated with Pierce's disease of grapes. Science 179:298-300.
5. HOPKINS, D. L., W. J. FRENCH, and H. H. MOLLENHAUER. 1973. Association of a rickettsia-like bacterium with phony peach disease. Phytopathology 63:443 (Abstr.).
6. HOPKINS, D. L., and J. A. MORTENSEN. 1971. Suppression of Pierce's disease symptoms by tetracycline antibiotics. Plant Dis. Rep. 55:610-612.
7. KING, E. O., M. K. WARD, and D. E. RANEY. 1954. Two simple media for the demonstration of pyocyanium and fluorescin. J. Lab. and Clin. Med. 44:301-307.
8. MISAGHI, I., and R. G. GROGAN. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent Pseudomonads. Phytopathology 59:1436-1450.
9. ZINSSER, H., F. FITZPATRICK, and H. WEI. 1939. A study of Rickettsiae grown on agar tissue cultures. J. Exp. Med. 69:179-190.