Mycoplasmalike Bodies Associated with Elm Phloem Necrosis

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ABSTRACT

Mycoplasmalike bodies were found associated with phloem cells in the roots and stems of American elms *(Ulmus americana)* infected by the elm phloem necrosis agent, but not in healthy elms. These bodies were limited by tripartite membranes (about 8 nm in width) and varied in diameter from 200 to 1,000 nm. They exhibited pleomorphic forms plus internal morphology typical of mycoplasmas. No virus particles were seen in phloem necrosis-diseased cells; however, numerous phloem elements were filled with plant protein.

Additional key words: virus diseases, trees.

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Phloem necrosis of elm was first described as a virus disease in 1938 after an investigation of numerous dying elms in Ohio by Swingle (12). Reports by Garman (8) and Forbes (7) suggest that this disease may have been present in the Ohio River Valley as early as 1882. A number of devastating epidemics was detected in the Midwest during the 1940's (4, 5, 6, 14, 15).

Elm phloem necrosis is still an important killer of American elm, *Ulmus americana* L., and winged elm, *Ulmus alata* L. We observed epidemics in northern Mississippi in 1970. While incidence of Dutch elm disease (DED) overshadowed the importance of phloem necrosis, recent progressive developments in the control of DED caused us to shift our attention to phloem necrosis because our remaining American elms are still in serious jeopardy.

McLean (10, 11) made a cytological comparison of phloem necrosis diseased and healthy elm tissue with the light microscope. He found that the fibrous roots die first after infection. The disease then progresses into the larger roots and finally, after death of the roots, the inner phloem in the lower portion of the stem may be killed. Hyperplasia and hypertrophy of parenchyma occurs in the phloem of older roots and the stem.

Swingle et al. (13) failed to see any difference between phloem exudates from diseased and healthy trees with the electron microscope. However, no detailed fine structure examination of elm phloem necrosis has been made.

The phloem necrosis agent is transmitted by a leafhopper (1, 2, 3), *Scaphoideus luteolus* Van Duzee, and once in the tree inhabits elm phloem. Yellows-type diseases that appear to be caused by mycoplasmas have the characteristics referred to above (9). Therefore, we initiated a cytological investigation to determine whether a mycoplasmalike organism is associated with elm phloem necrosis.

MATERIALS AND METHODS.—Phloem necrosis-diseased and healthy tissues from *U. americana* were collected near Stoneville, Miss., and Worthington, Ohio. Diseased tissue from the stem and roots showed typical symptoms of elm phloem necrosis, a butterscotch color and wintergreen odor. Healthy phloem was creamy white and did not emit a wintergreen odor. Inner phloem from the stem and large roots was minced with a razorblade and fixed in 3% glutaraldehyde in phosphate buffer at pH 6.8 for 12 hr. The tissue was then washed in pH 6.8 phosphate buffer and postfixed in 2% osmium tetroxide (phosphate buffered to pH 6.8) for 2 hr. It was then dehydrated in ethanol and placed in propylene oxide to which Epon mixture was added as the propylene oxide-Epon mixture was drawn off. Finally, the tissue was placed in pure Epon and then into capsules. Embedded tissues were sectioned on an LKB ultratome with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Hitachi HU-11E electron microscope.

RESULTS.—Mycoplasmalike bodies ranging in size from 200 to 1,000 nm were abundant in certain root and stem phloem elements of trees infected with elm phloem necrosis (Fig. 1-A, B; 2). Phloem in infected tissue was often filled with a dark-staining matrix which made it difficult to determine the nature of inclusions (Fig. 1-C). Vesicular bodies superficially resembling mycoplasmas were also evident in healthy tissue, but lacked the distinctive internal morphology of mycoplasmas. Degenerating mitochondria and polyvesicular bodies in healthy tissue often resemble mycoplasmas. The lack of distinctive internal morphology in the polyvesicular bodies and the presence of degenerating cristae in the mitochondria helped identify these bodies.

The mycoplasmalike bodies in diseased tissue were primarily spherical or oval, but filamentous forms were also evident (Fig. 2-A). A distinct tripartite membrane was seen in certain sections averaging about 8 nm in thickness (Fig. 2-D). A clear area in the center of some mycoplasmalike bodies had a reticulum suggesting a nucleoid (Fig. 1-B).

Considerable pleomorphism was exhibited by the mycoplasmalike bodies in diseased phloem (Fig. 2). Spherical bodies with filamentous extensions and small blebs were common. Some forms apparently budded to form a beaded arrangement (Fig. 2-A, B).

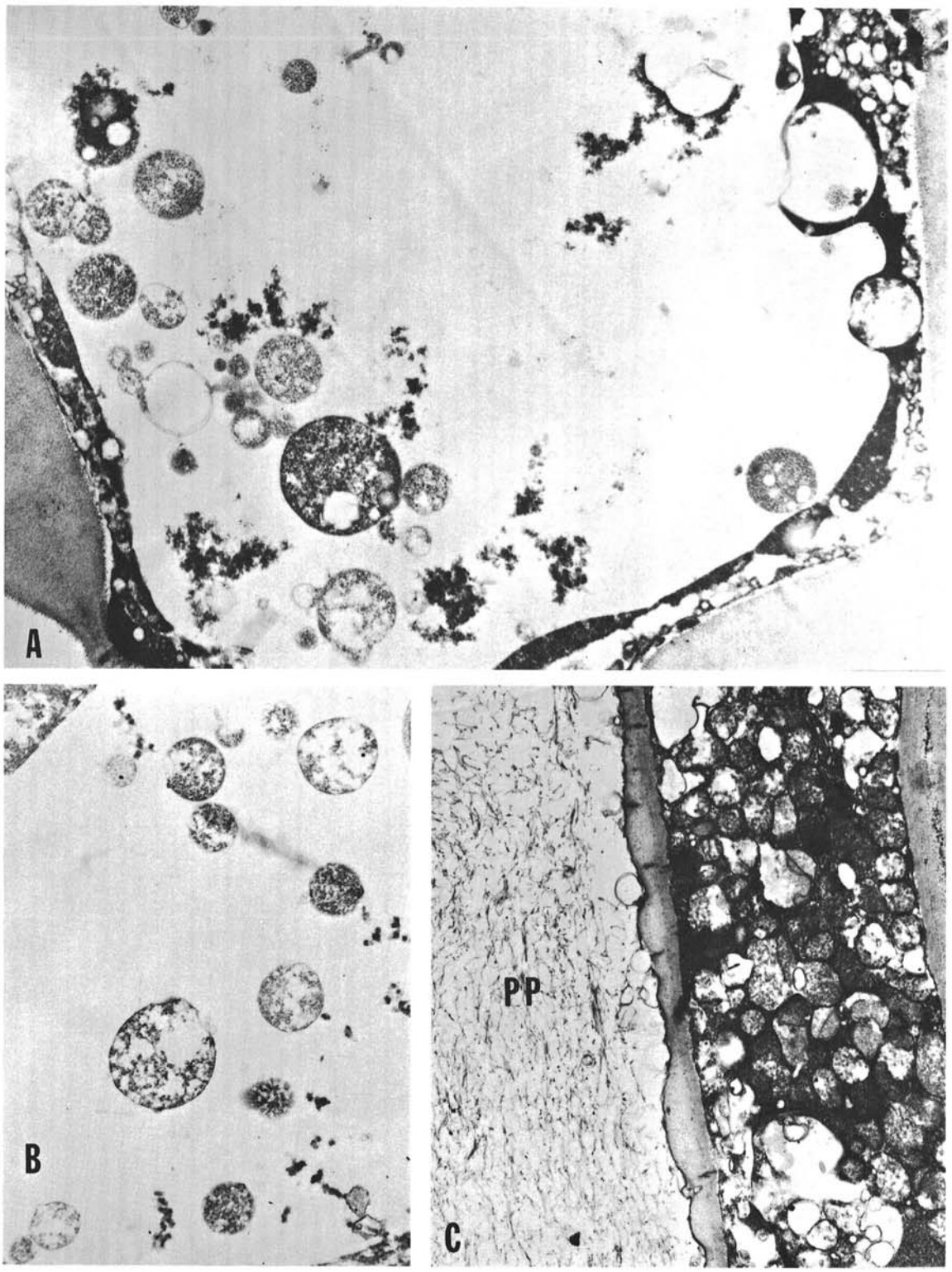

Fig. 1. Ultrathin sections of root phloem of American elm with phloem necrosis. **A)** Cross section of phloem element with mycoplasmalike inclusions (X ca. 16,600). **B)** Mycoplasmalike bodies in phloem element (X ca. 15,000). **C)** Longitudinal section through two phloem elements, one filled with dark-staining matrix containing vesicular and mycoplasmalike bodies and the other, plant protein (PP) (X ca. 9,800).

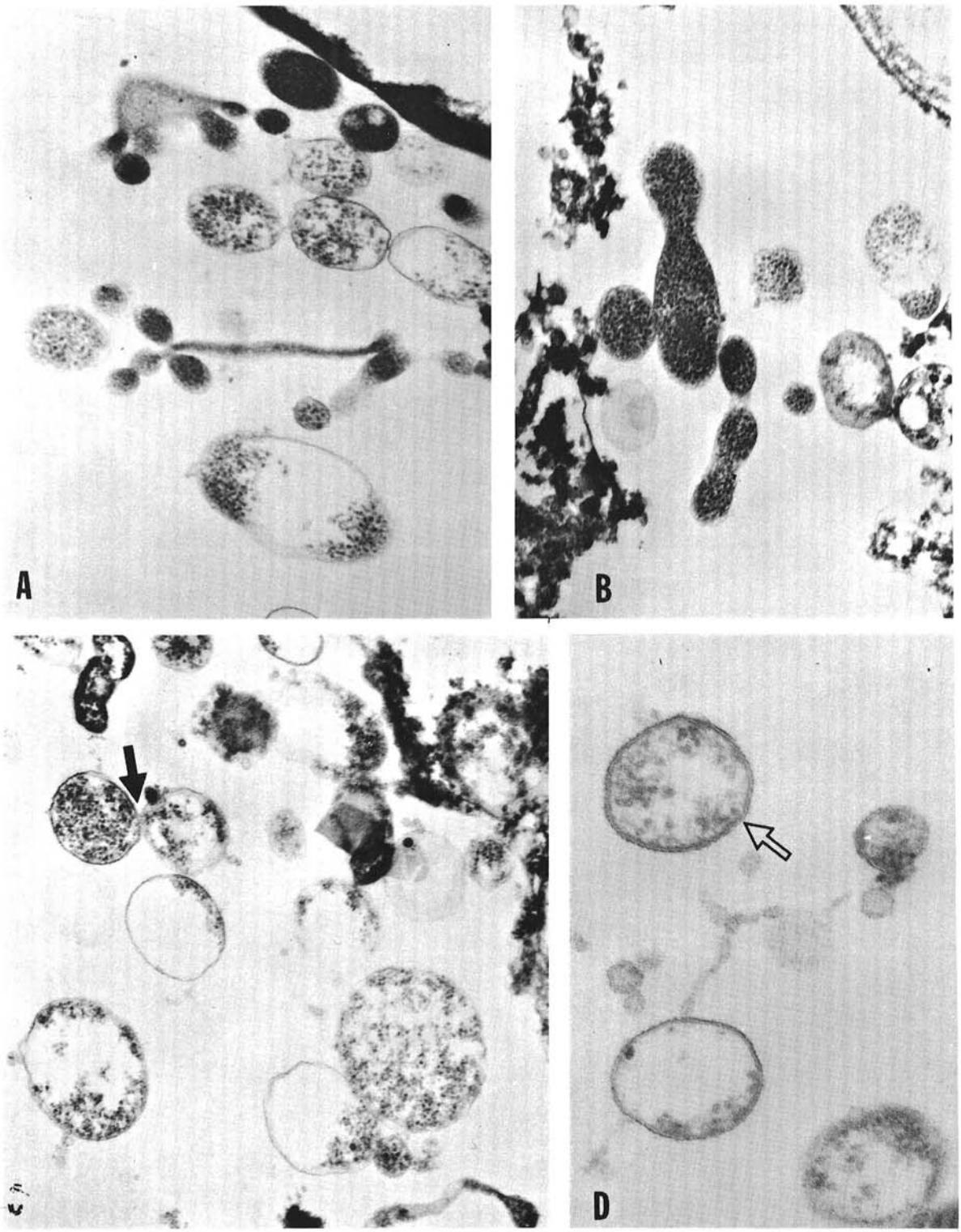

Fig. 2. Morphology of mycoplasmalike bodies in phloem elements of elm. **A)** Filamentous and dendroid forms. Note budlike growth (X ca. 38,000). **B)** Chainlike arrangement (X ca. 20,750). **C)** Filament and spherical forms. Note suggestion of binary fission (arrow) (X ca. 40,000). **D)** Mycoplasmalike bodies with distinct tripartite membrane (arrow) (X ca. 95,500).

There was a suggestion that some bodies were in the process of dividing by binary fission (Fig. 2-C, arrow).

Large dark-staining bodies were formed characteristically in mitochondria of infected phloem cells from stem and root tissue, but not in degenerating mitochondria in phloem cells of healthy trees. They were delimited by a distinct tripartite membrane, and were freed into the cell lumen upon disintegration of the mitochondria. No virus particles were observed in phloem elements. However, many infected cells were filled with plant protein (Fig. 1-C).

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