Fine Structure of Ceratocystis ulmi in Elm Wood

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Accepted for publication 10 May 1972.

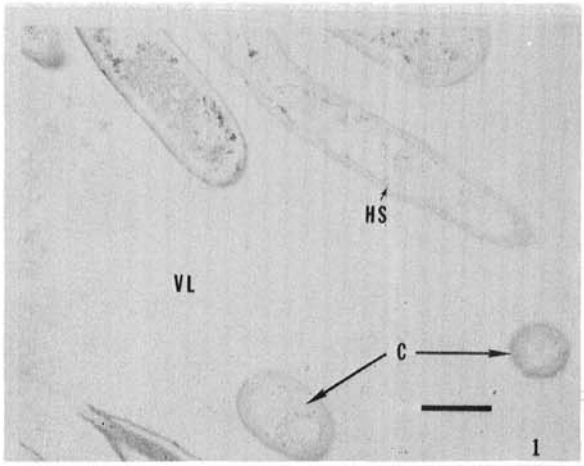
ABSTRACT

Direct penetration of vessel cell walls and invasion of peritracheal parenchyma cells of *Ulmus americana* by *Ceratocystis ulmi* were observed 2 weeks after inoculation. An appressoriumlike structure was apparent when hyphae penetrated pit membranes. A dark-staining area was formed around hyphae in response to invasion of parenchymal cells. *C. ulmi* caused little over-all damage to cell walls.

Phytopathology 62:1253-1256.

The distribution of the Dutch elm disease fungus, *Ceratocystis ulmi* (Buis.) C. Moreau, in host tissue has been investigated since this disease was first recognized. After more than 40 years of research, there is still disagreement about fundamental aspects of fungal colonization and parasitism. Controversy exists over whether the parenchymal tissue is invaded (6), whether "microhyphae" and "microendospores" are formed (4, 5), whether plugging by the fungus and/or gums and tyloses is important in disease development (7), and whether the fungus is able to penetrate cell walls directly (3).

Early workers had difficulty distinguishing fungal elements in diseased elm tissue with the light microscope. Pomerleau (6) has recently reviewed those studies using the light microscope. He rejects all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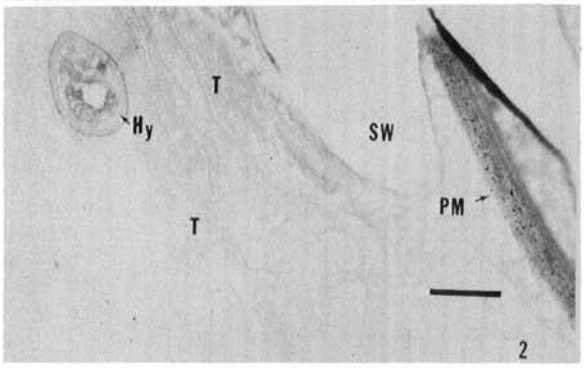
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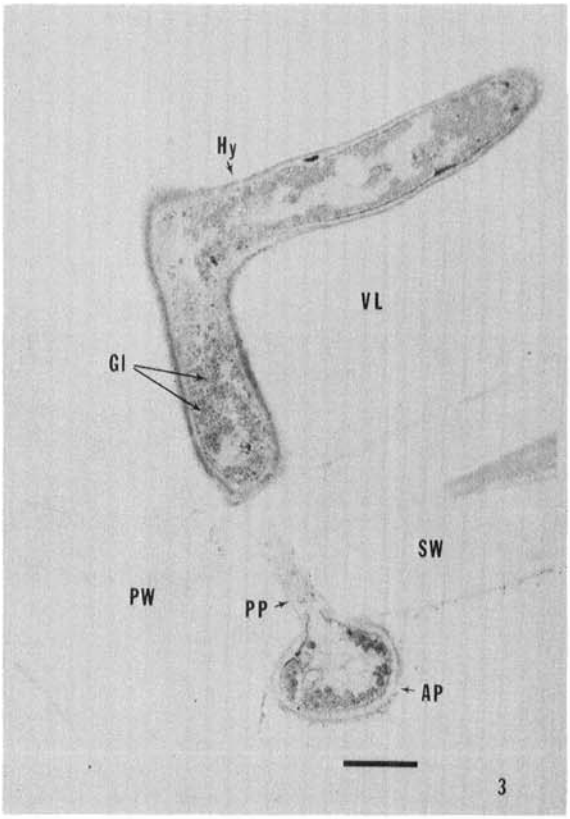
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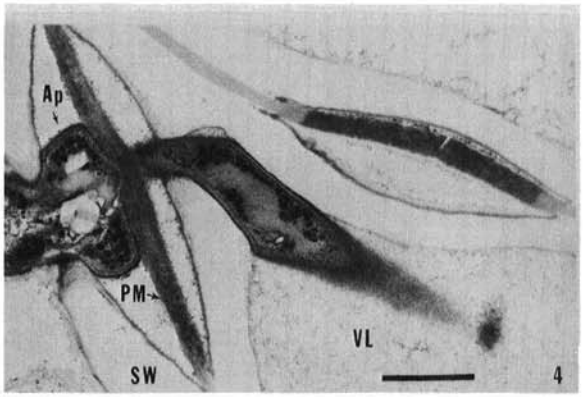
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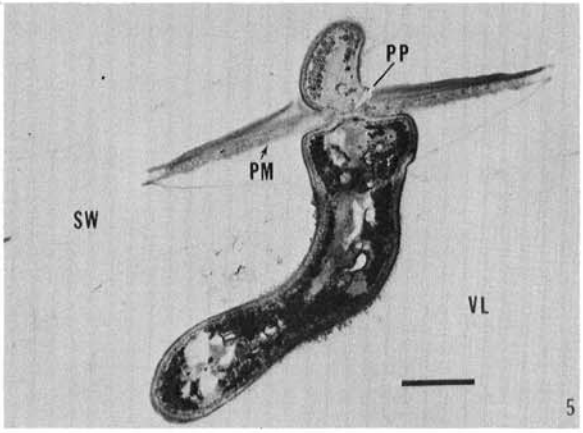
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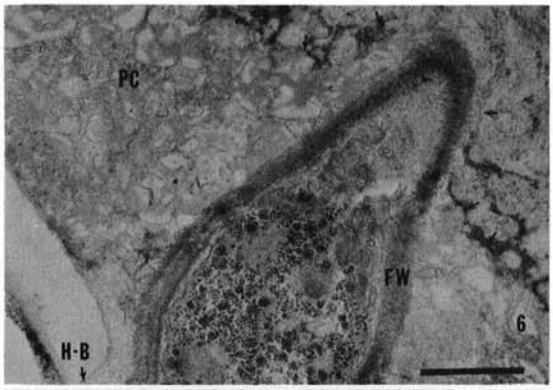
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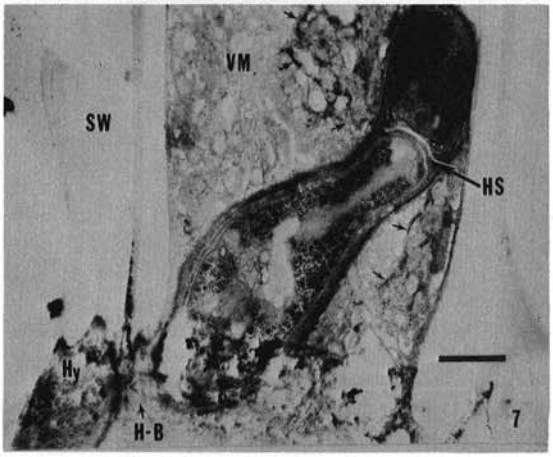
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reports of the presence of the fungus in parenchymatous tissue, contending that earlier workers were observing secondary organisms.

Fine structure observation with the electron microscope allows a clearer visualization of the interaction of the host and parasite, and provides the opportunity to resolve some points of controversy. Recently MacDonald & McNabb (3) were able to show direct penetration of vessel elements by *C. ulmi* in electron micrographs. MacDonald (2) and Jones (1) reported fine structure studies of *C. ulmi* in elm wood.

It was the purpose of this study to investigate host-parasite interaction in Dutch elm disease with the electron microscope. Special attention was given the invasion of parenchyma, since Pomerleau (6) has stated so unequivocally that parenchymatous tissue is not invaded.

MATERIALS AND METHODS.–Eight 3-year-old seedlings of *Ulmus americana* L. were inoculated with conidial suspensions of *C. ulmi*. Samples of sapwood were taken 12 inches above the point of inoculation 1, 2, 3, and 4 weeks after inoculation. Each sample was taken from previously unsampled seedlings. Similar samples were cut from noninoculated seedlings. The fresh tissue was immediately fixed in 3% glutaraldehyde in phosphate buffer at pH 6.8 for 18 hr. The tissue was then washed in pH 6.8 phosphate buffer, postfixed in 2% osmium tetroxide (phosphate buffered to pH 6.8) for 2 hr, and washed in pH 6.8 phosphate buffer. It was dehydrated in ethanol, placed in propylene oxide, then in a mixture of propylene oxide and Epon. Finally, the tissue was placed in pure Epon and then into capsules. The capsules were placed in ovens for 24 hr each at 30, 45, and 60 C. Embedded tissues were sectioned on an LKB ultratome with a diamond knife, poststained with uranyl acetate for 45 min and lead citrate for 1 min, and examined with a Hitachi HU-11E electron microscope.

RESULTS.–Some inoculated seedlings began to show foliar symptoms of DED 3 weeks after inoculation, and all were symptomatic by the 4th week. Xylem discoloration was observed in the 1st week after inoculation.

Hyphae and spores of *C. ulmi* were observed in xylem vessels 1 week after inoculation (Fig. 1). The presence of spores was determined by following serial sections. Tyloses and gums were also present in vessels. Generally tyloses were not found in vessels containing hyphae and spores (Fig. 1); in some instances, however, they were associated with the presence of the pathogen (Fig. 2).

Direct penetration by the fungus through vessel cell walls was observed from tissue sampled 2 weeks after inoculation (Fig. 3). Hyphae also penetrated through the pit membrane of bordered pits (Fig. 4). There were suggestions that an appressoriumlike

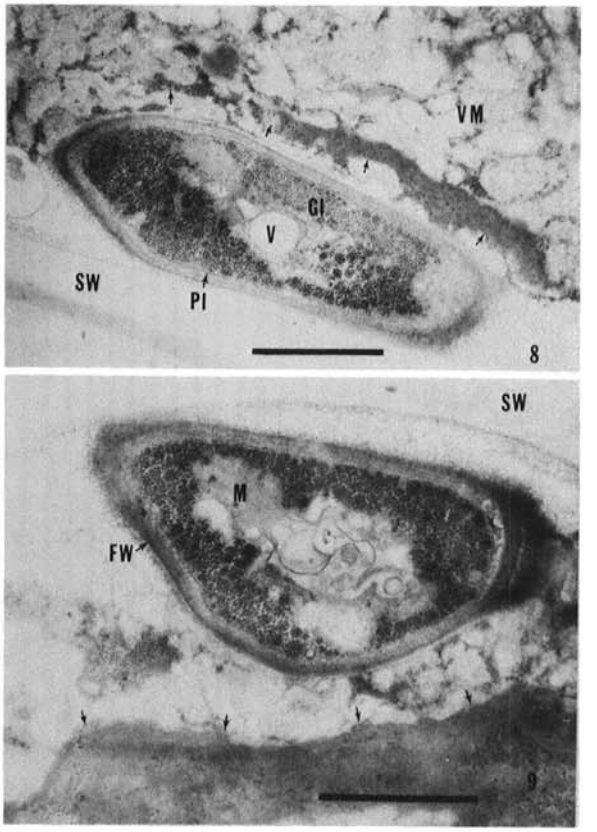

Fig. 8.-9. Scale = 1 μ. **8)** Cross-section of *Ceratocystis ulmi* hypha in a parenchymal cell. The fungal plasmalemma, glycogen bodies, and a vacuole are seen within the hypha. In the host cell adjacent to the hypha, there is a dark-staining area (arrows). **9)** A sequential section of the cell from Fig. 8; arrows designate host reaction.

Fig. 1.-7. Scale = 1 μ. **1)** Hyphae and conidia of *Ceratocystis ulmi* in a xylem vessel 1 week after inoculation. Note the hyphal septum. Ap = appressoriumlike structure; C = conidia; FW = fungal cell wall; G1-glycogen bodies; H-B = half-bordered pit; Hy = hypha; HS = hyphal septum; M = mitrochondrion; PC = parenchymal cell; P1 = fungal plasmalemma; PM = pit membrane; PP = penetration peg; PW = primary cell wall; SW = secondary cell wall; T = tyloses; V = vacuole; VL = vessel lumen; VM = vesicular matrix. **2)** A hypha, in cross-section, is present in a xylem vessel adjacent to tyloses. **3)** Hypha directly penetrating the cell wall of a vessel. Note the appressoriumlike structure and penetration peg of the hypha as well as glycogen bodies within the hypha. **4)** *C. ulmi* penetrating an intertracheary pit membrane by means of an appressoriumlike structure. **5)** Pit membrane is forced against the side of an intertracheary pit pair by the appressoriumlike structure of a *C. ulmi* hypha. A hyphal penetration peg seems to push through the membrane. **6)** Invasion of a xylem parenchymal cell by *C. ulmi* through a half-bordered pit. Arrows point to a dark-staining area near the hypha which appears to be a localized host response. **7)** Invasion of *C. ulmi* hypha from xylem vessel through a half-bordered pit into a parenchymal cell. Note the septum of the invading hypha and the darkly stained area (arrows), and the vesicular matrix of the host cytoplasm.

structure was formed from which a small hyphal peg penetrated through the primary wall (Fig. 4, 5).

Peritracheal parenchymatous cells were invaded through half-bordered pits from xylem vessels in tissue sampled 2 weeks after inoculation (Fig. 6). Large hyphae developed in the parenchyma cell, and host cell contents were transformed into a vesicular matrix (Fig. 7).

There appeared to be a localized host response to hyphae in parenchymatous cells. A dark-staining area was apparent near hyphae (Fig. 8, 9). Whether this is an area of greater transformation or a host response to invasion could not be determined.

There appears to be little over-all damage to vessel and parenchymatous cell walls by *C. ulmi*. The most prominent response is a darkening of the primary wall of pit membranes between bordered pits (Fig. 2). Additional vascular discoloration is attributed to vacuolar, tanninlike inclusions within parenchymatous cells.

Vascular dysfunction has been presented as the primary cause of Dutch elm disease. This conclusion has recently been questioned (7). There does not appear to be sufficient digestion of cell wall materials to account for changes in water relations in the tree. Besides the production of gums and tyloses, the most apparent disruptive response to *C. ulmi* is necrosis of the parenchyma and a darkening of the pit membranes in bordered pits.

Response of the parenchymatous tissue to invasion by *C. ulmi* is interesting. The host cytoplasm appears to respond to fungal invasion by the deposition of dark-staining materials at the forefront of encroachment. Such areas may be sites of tranformation by the fungus or they may be resistant responses by the host.

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