Ultrastructure at the Host-Parasite Interface of Phytophthora megasperma var. sojae in Soybean Rootlets

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ABSTRACT

Ultrastructure and reactions at the host-parasite interface of *Phytophthora megasperma* var. *sojae* in soybean roots were studied. The inter- and intracellular penetration of host cells and tissues by hyphae and the formation of haustoria is described. Evidence for degradation or dissolution of host walls is presented. Lomasomes differed in structure from other *Phytophthora* spp., and a previously undescribed separation between host cytoplasm and host wall was found in areas of fungal contact. Two partially delineated zones within the extrahaustorial matrix are shown. The haustorial wall differed from the hyphal wall by its greater thickness, presence of an outer, dark-staining zone containing electron-dense inclusions, and an outermost granular boundary.

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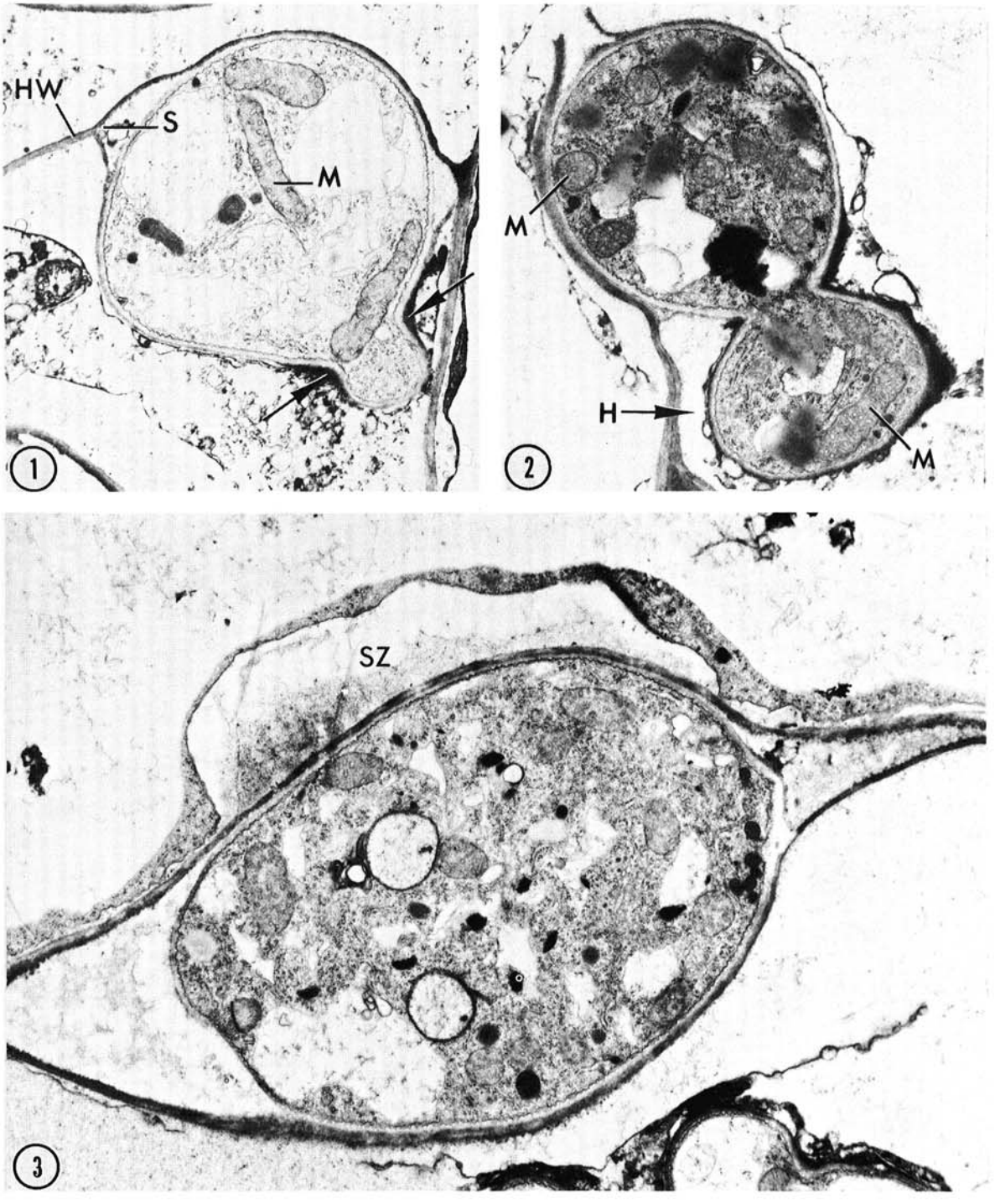
Intergeneric and interspecific comparisons have been made of the ultrastructure of host-parasite interfaces of haustoria-forming fungal parasites. These studies have shown encapsulated, sheathed and nucleated haustoria, septate haustorial necks, dictyosomes, and lomasomes (4). The only consistent feature was an encapsulated haustorium. Studies of the host-parasite interfaces of *Phytophthora* spp. have been confined to *P. infestans* and *P. parasitica* (5, 6). An abstract of an electron microscope study reported haustoriumlike bodies of *P. megasperma* var. *sojae* (*Pms*) in soybean hypocotyls (8). We report on ultrastructure studies of haustoria and reactions at the host-parasite interface of *Pms* in soybean rootlets.

MATERIALS AND METHODS.–*Phytophthora megasperma* Drechs. var. *sojae* Hild. Race 1 (10) (*Pms*) was isolated from diseased soybeans [*Glycine max* (L.) Merr.] collected in Champaign county. Illinois, and maintained on V-8 juice + $CaCO_3$ agar (9). Inoculum (mycelium, sporangia, and zoospores) was prepared by cutting (with a sterile cork borer) 5-mm diam disks from the edge of an actively growing *Pms* colony, transferring a single disk to each of four beakers containing 75 ml sterile, distilled water, and incubating at 25 C. The original water was replaced with fresh sterile water at 14 and 15 h after inoculation.

Soybean (susceptible to *Pms*) seeds (cultivar Amsoy) were surface sterilized by immersion in 2.75% sodium hypochlorite solution for 3 min, then in 70% ethanol for 1 min, followed by a sterile distilled water rinse. All seeds were air-dried before planting in moist, sterile vermiculite (Terralite) and grown for 5 days in a controlled environmental chamber programmed for 32 C, 50% relative humidity and at a 14-h day of 86,080 lx (8,000 ft-c). The seedlings were removed, washed in sterile water, and one seedling was placed in each beaker containing either the *Pms* suspension or sterile water (control) so that the root systems were completely immersed for 3 days. Root tips were examined under a light microscope for the presence of *Pms*.

For electron microscope studies, 4-6 mm segments of roots with and without *Pms* were fixed for 2-4 h at room temp (25 ± 3 C) in 4% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.2. The segments were then aspirated for 15 min at 800 mm Hg to facilitate



Legend: EM = extrahaustorial matrix; FPL = fungal plasmalemma; FW = fungal wall; GL = granular layer; H = haustorium; HC = host cytoplasm; HPL = host plasmalemma; HT = host tonoplast; HW = host wall; IL = inner layer; IN = inclusion; L = lomasome; M = mitochondrion; OL = outer layer; S = separation; SZ = separation zone; and V = vacuole.

Fig. 1-3. **1)** Soybean seedling rootlets infected with *Phytophthora megasperma* var. *sojae*. Hyphal penetration of necrotic cell showing separation at middle lamella, papillum-like material (arrows), and early stage of haustorial development (× 11,700); **2)** intercellular hyphal growth with haustorial penetration of adjacent cell (× 13,700); **3)** Separation zone in host cell developing opposite contact area of intercellular hypha (× 13,100).

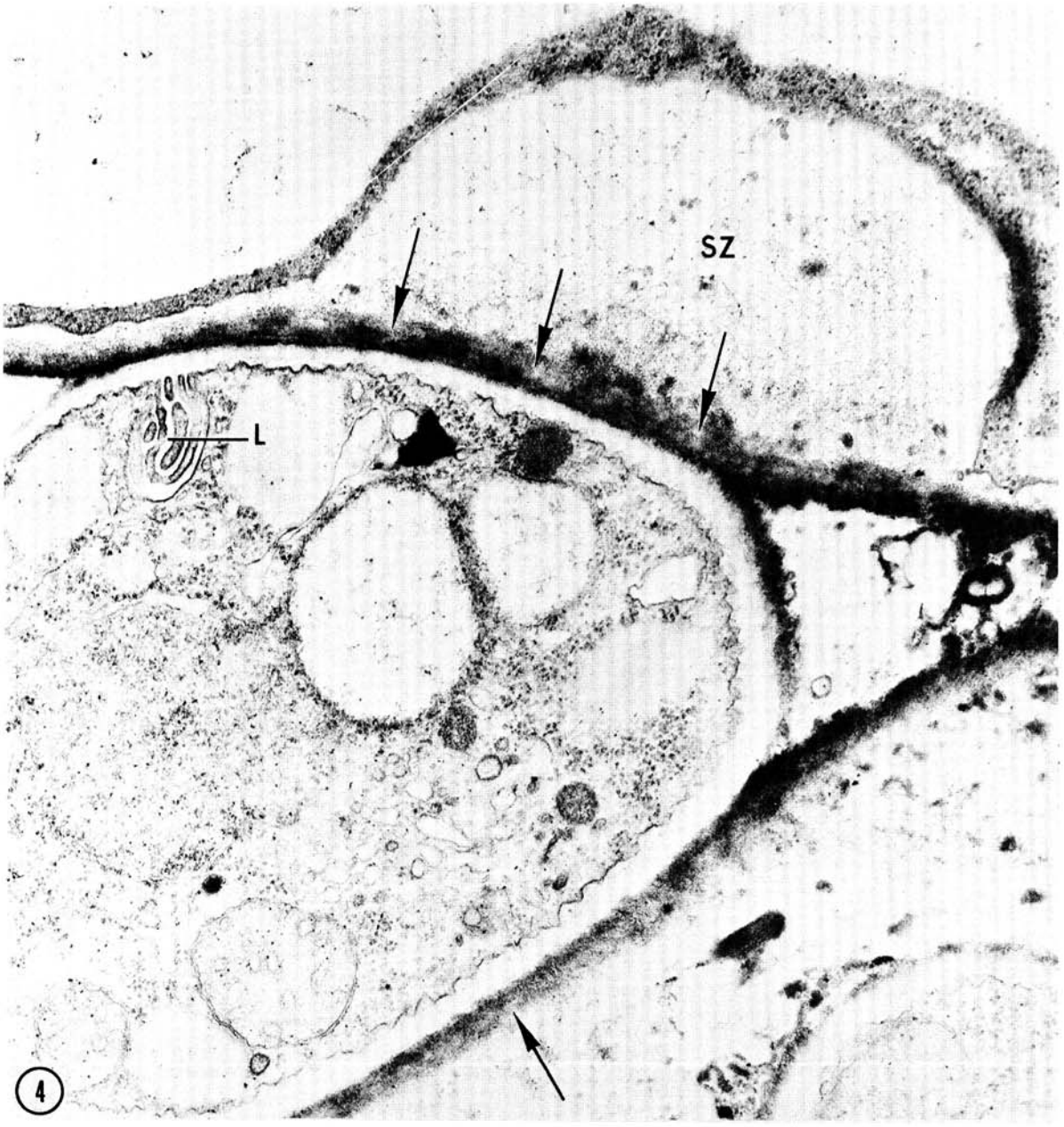
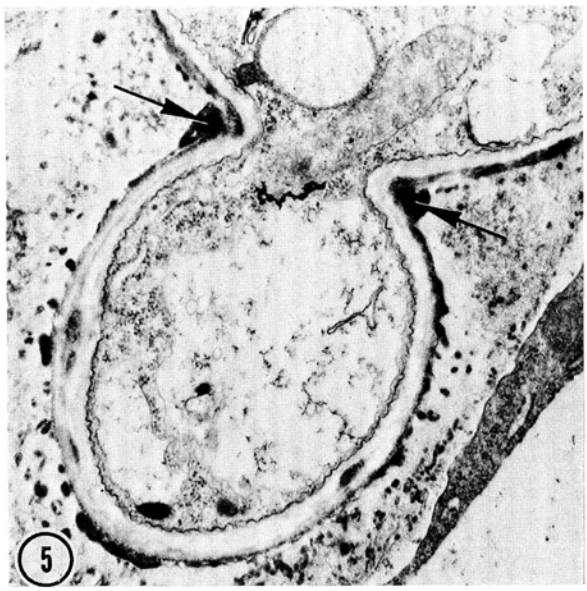


Fig. 4. Soybean seedling rootlets infected with *Phytophthora megasperma* var. *sojae*. Intercellular hyphae showing separation zone in host cell and areas of apparent host wall degradation (arrows) (× 35,400).

penetration of the fixative, washed in several changes of the buffer, and postfixed in 2% OsO_4 in the same buffer. Following dehydration in a graded ethanol series, the segments were transferred through propylene oxide and embedded in Epon 812. Thin-sections were cut with glass knives on a Sorvall MT-2 ultramicrotome and mounted on 200-mesh copper grids. Sections were stained for 5 min in 3% aqueous uranyl acetate, and poststained for 3 min in lead citrate. Observations were made with a Hitachi HU-11a electron microscope.

RESULTS AND DISCUSSION.—*Fungal fine*

Fig. 5-7. 5) Soybean seedling rootlets infected with *Phytophthora megasperma* var. *sojae*. Haustorium with papillumlike material (arrows) at penetration zone (× 22,800); **6)** intercellular hyphal growth separating host cell primary wall from middle lamella (× 10,600); **7)** intercellular hypha with haustorium showing inner and outer layer of extrahaustorial matrix, and thickened haustorial wall (× 18,900).



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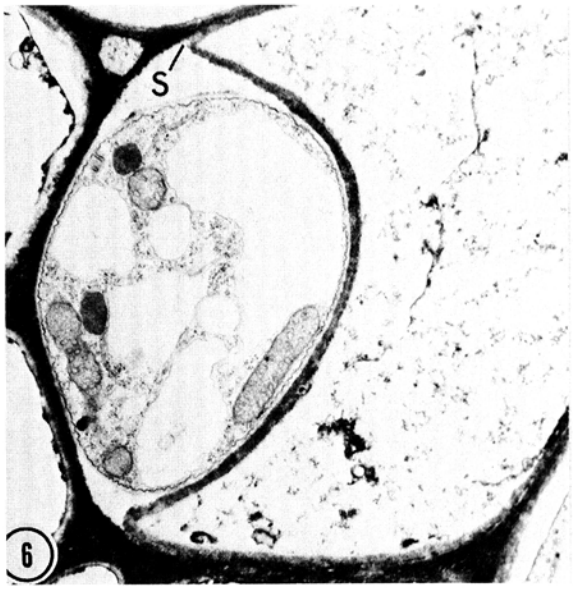

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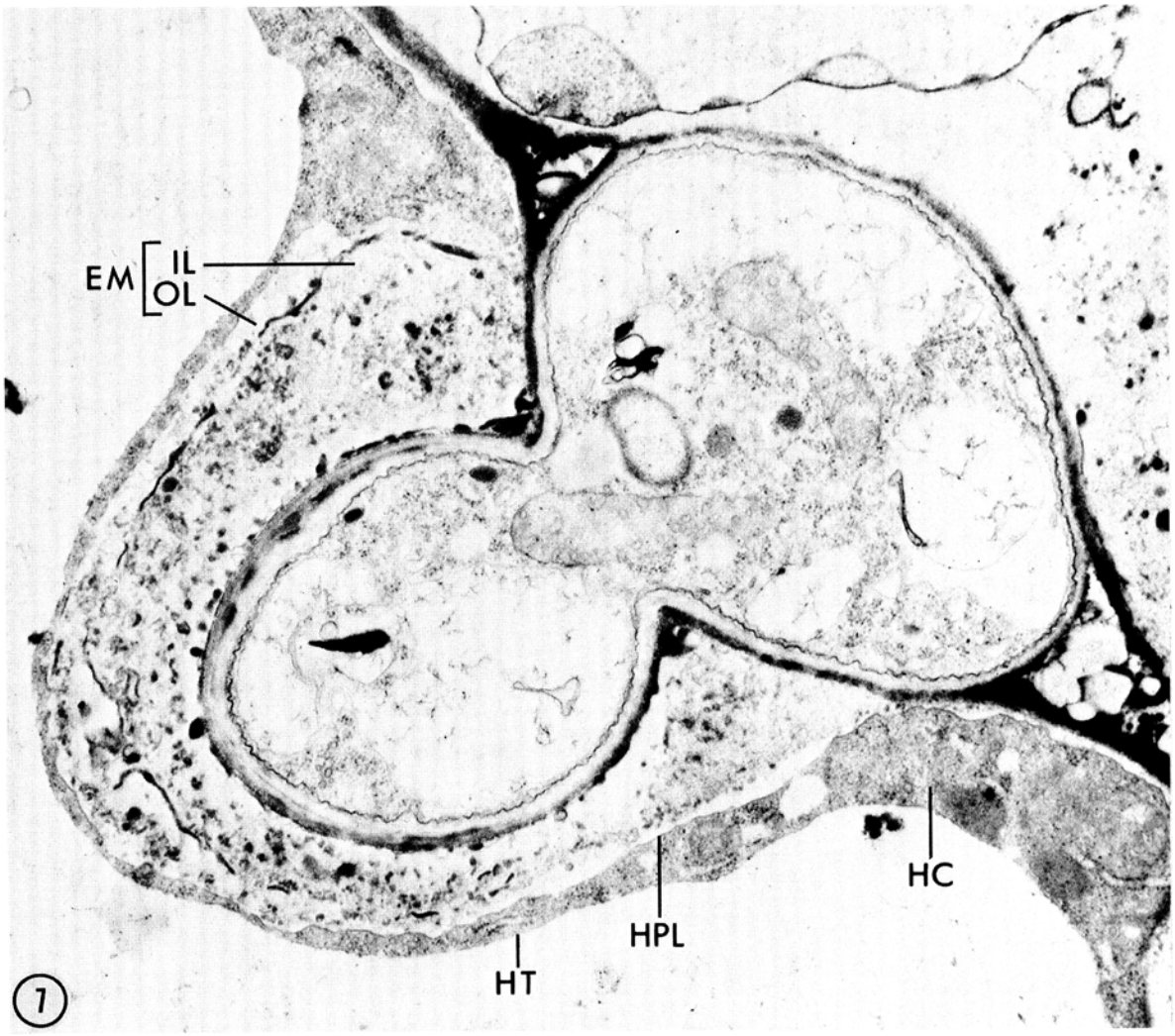

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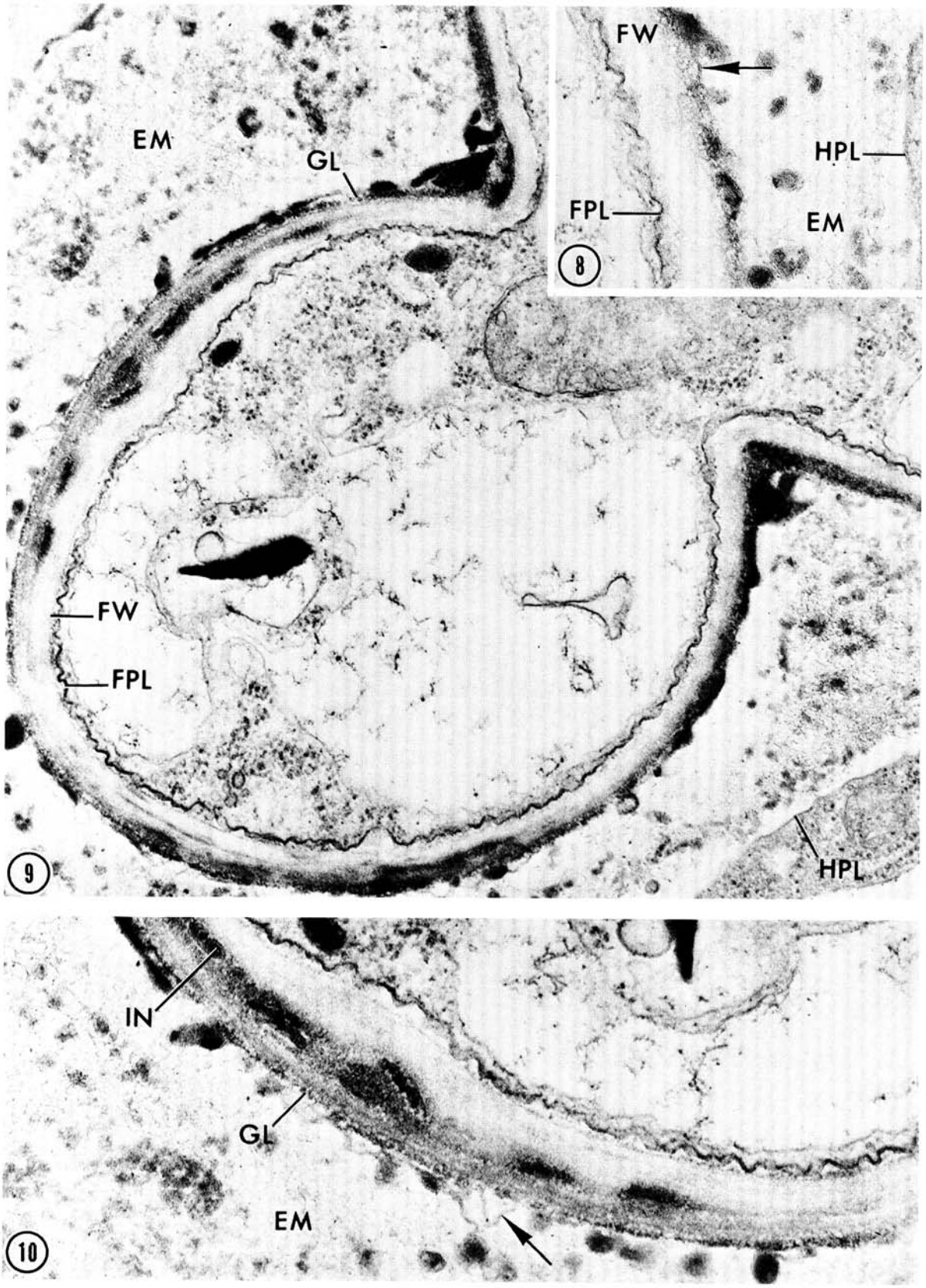
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structure.—Endoplasmic reticulum, ribosomes, vacuoles, and lipoidal vacuolar inclusions were abundant throughout the hyphae. Haustoria were found penetrating both necrotic (Fig. 3) and living (Fig. 9) host cells. There was no evidence of cell necrosis in advance of the hyphae. Fungal mitochondria tended to be oriented parallel to the hyphal long axis in both inter- and intracellular hyphae (Fig. 3), a condition not apparent in cells infected by *P. infestans* (4). The plasmalemma, which appeared undulant in hyphal trans- and longisections (Fig. 9). was found to be rugulose in highly oblique sections. The lomasomes were larger, less complex, and have smaller openings (Fig. 6) than those of *P. infestans* (4) and *Peronospora manshurica* (11) and resembled those of *Puccinia sorghi* (12).

Intercellular host-parasite relations.—Intercellular hyphae generally were observed to grow either between adjacent cells, separating the cells at the middle lamella (Fig. 1), or between the middle lamella and cell wall (Fig. 8). A previously undescribed separation of cytoplasm from the host cell wall was observed when hyphae lay appressed to the host cell wall (Fig. 4, 6). The host cell wall in the zone of cytoplasmic separation appeared affected by uneven staining. This may represent a reaction induced during an early phase of haustorium formation. A zone similar in appearance was reported during the early stages of papilla formation by *Erysiphe graminis* in barley (3).

Host cell penetration and haustorium structure.—Papillum development is a normal, nonspecific host response to wounding (2). Bracker and Littlefield include papilla as a type of host cell wall apposition (1). Papillar-like material was observed at the penetration pore (Fig. 7, 9), but not inside the host cell. Papillum formation was described for *Erysiphe graminis* (1), *Peronospora parasitica*, and other fungi (2). Hanchey and Wheeler (6) refer to "wall lesions" in cells attacked by *Phytophthora parasitica* var. *nicotianae*, which were later described by Bushnell as resembling papillae.

An extrahaustorial matrix (2), usually referred to as sheath, is produced between the haustorium and the host cytoplasm, and it constitutes a boundary across which material may be transported between host and pathogen (9). In *Pms* this resembles a heterogenous mantle surrounding the haustorium and includes granular and finely fibrillar material (Fig. 9, 10, 9). The diffuse material in the separation zone (Fig. 4) may also be later incorporated in the extrahaustorial matrix. A typical sheath is found in *P. infestans.* The sheath of *P. parasitica* differs in its lack of inclusions and apparent continuity with the cell wall (6). The composition of sheaths and papillae is still undetermined. The most plausible origin is from mutual secretion at the host-pathogen interface (1).

In *Pms*, the haustoria resemble those of *P. infestans* (4) and showed a scarcity of organelles in the haustorial head. The wall of the *Pms* haustorium, however, was generally thicker than the hyphal wall (Fig. 9) and suggested greater structural complexity. The outer half of the wall had an ill-defined, laminate structure and stained unevenly. Outermost was a thin, rather granular layer which was in contact with the extrahaustorial matrix (Fig. 9, 10). Between this granular layer and the midpoint of the wall was a darker staining zone containing denser inclusions of an undetermined nature (Fig. 10). The extrahaustorial matrix itself had a zonate organization (Fig. 9). A relatively thick inner layer of heterogeneous material was delimited from a thinner, outer layer of lightly staining material by a discontinuous, dark boundary.

There is considerable evidence to indicate that the extrahaustorial membrane, though continuous with the host plasma membrane, has structural and chemical properties that indicate some type of tissue. specialization at the host-haustorium interface (1). Hardwick et al. (7) hypothesized that variations in the morphology of different parts of the newly formed membrane of the invaginated host protoplast may be assumed to be consequences of changes which occur in the interaction of host and parasite (7). Since stretching of an existing membrane is inadequate to form the extrahaustorial membrane, mechanisms have been suggested to account for the increased surface area (1). In this study, however, there was no apparent structural specialization of the host plasma membrane in the area of association with the haustorium. Rather, the host plasmalemma appeared to be continuous over the periphery of the matrix zone. The few membranous fragments in the inner matrix close to the haustorial wall (Fig. 10), are likely derived from bound, osmiophilic inclusions.

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Fig. 8-10. 8) Soybean seedling rootlets infected with *Phytophthora megasperma* var. *sojae*. Portion of extrahaustorial matrix between host plasmalemma and fungal wall; arrow indicates membranous fragment (× 65,900); 9) enlarged portion of haustorium shown in Fig. 7 (× 35,600); 10) enlarged portion of Fig. 7 showing details of haustorial wall structure; arrow indicates membranous fragments in inner layer of extrahaustorial matrix (× 62,400).

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