Histopathology of Resistant and Susceptible Soybean Hypocotyls Inoculated with Phytophthora megasperma var. sojae

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ABSTRACT

Hypocotyls of resistant and susceptible soybean plants were examined by light and electron microscopy 3 days after inoculation with *Phytophthora megasperma* var. *sojae*. All tissues of susceptible hypocotyls were ramified by both inter- and intracellular hyphae of the pathogen. Parenchyma near the site of infection was completely disorganized, but vascular tissues and cells with secondary walls remained intact even when heavily infected. In inoculated hypocotyls of resistant plants only those cells directly surrounding the inoculation wound were colonized by the pathogen, and normal appearing organelles were present in adjacent noninfected host cells. Some host cells close to the infected area were filled with granular, dark-staining cytoplasm which appeared to form a barrier to further movement of the fungus.
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Additional key words: Glycine max, fine-structure.

Soybean cultivars Harosoy (H) and Harosoy 63 (H63) are nearly isogenic – differing only by the single gene in H63 that confers resistance to the soybean pathogen, *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildb. (*Pms*) (1). When *Pms* mycelium is inserted into hypocotyls of resistant seedlings, hypersensitive reactions occur around inoculation wounds and no additional symptoms develop. Similar susceptible plants, however, develop watersoaked lesions within 48 h after inoculation, and after 72 h, hypocotyls are completely rotted (7).

The difference in the reactions of the two cultivars when inoculated with *Pms* has been ascribed to different rates of production of phytoalexin at the site of infection (4,6). The phytoalexin, which has been identified as 6-α-hydroxyphaseollin, accumulates in the inoculation wounds of H63 hypocotyls 10 to 100 times faster than in those of H plants (6). In this paper, we compare by light and electron microscopy the histopathology of H and H63 hypocotyls inoculated with *Pms*.

MATERIALS AND METHODS.–Hypocotyls of 8-day-old soybeans, *Glycine max*. (L.) Merr. 'Harosoy' and 'Harosoy 63' were wound-inoculated with mycelium from 7- to 9-day-old cultures of *Pms* grown in hempseed and water medium (7). Three days after inoculation, 3-cm-long hypocotyl-sections from the area of the inoculation site were removed and prepared for microscopic examination. Comparable tissues from uninoculated, but wounded, soybean plants served as controls.

Sections to be examined by light microscopy were trimmed to ca. 2 cm, submersed in Belling's Modified Navaschin's fixative for 24 h, dehydrated in a standard tertiary-butyl-alcohol series, and embedded in paraffin (5). Ten-micron serial sections were cut with a rotary microtome, mounted on slides, and stained with safranin and fast green. Sections were examined with a Zeiss research microscope, and photographs were made with Panatomic-X film.

Sections to be used for electron microscopy were fixed in Millonig's buffered gluteraldehyde (9) and postfixed in 1% buffered osmium. The tissue pieces were dehydrated in a graded series of ethyl alcohol, embedded in Maraglas-Cardolite (3), and sectioned with a diamond knife in a Porter-Blum MT-1 microtome. Ultrathin sections were stained with uranyl acetate and lead citrate (10), and examined in a Hitachi HU-11C-1 electron microscope.

RESULTS AND DISCUSSION.–In inoculated hypocotyls of susceptible H plants, disorganization of the parenchyma near the site of infection was almost complete in 3 days. Cells were generally devoid of cytoplasm and their walls were either broken or collapsed (Fig. 7). Host cells with secondary walls, such as fiber cells, however, remained intact even when heavily infected (Fig. 9). Xylem vessels also remained intact even though they were often filled with mycelium (Fig. 2). Vascular tissues seemed to be preferentially invaded by *Pms* mycelium which extended beyond the area of the hypocotyl where the fungus colonized other tissues. Disorganization of host tissues became less severe away from the site of inoculation, but *Pms* hyphae were present in tissues which were only slightly disorganized indicating little degradation of host tissues far in advance of the pathogen.

Hyphae of *Pms* often grew intercellularly in H hypocotyls (Fig. 1, 10), but intracellular hyphae were abundant (Fig. 2, 3, 7, 8, 9). Hyphae growing in parenchymatous tissues (Fig. 1) were considerably larger in diam than those growing in vascular tissues (Fig. 2, 3). Hyphae were constricted at the points where they passed through the host cell wall (Fig. 8, 10). Although haustoria-like bodies were sometimes observed (Fig. 10), the high frequency of intracellular hyphae suggested that these bodies were not true haustoria but hyphae which had recently penetrated the host cell wall.

In inoculated hypocotyls of resistant H63 plants, there was no disorganization of tissues except for those directly surrounding the inoculation wound. Where *Pms* hyphae were observed, they were both intercellular (Fig. 11) and intracellular (Figs. 6, 12,

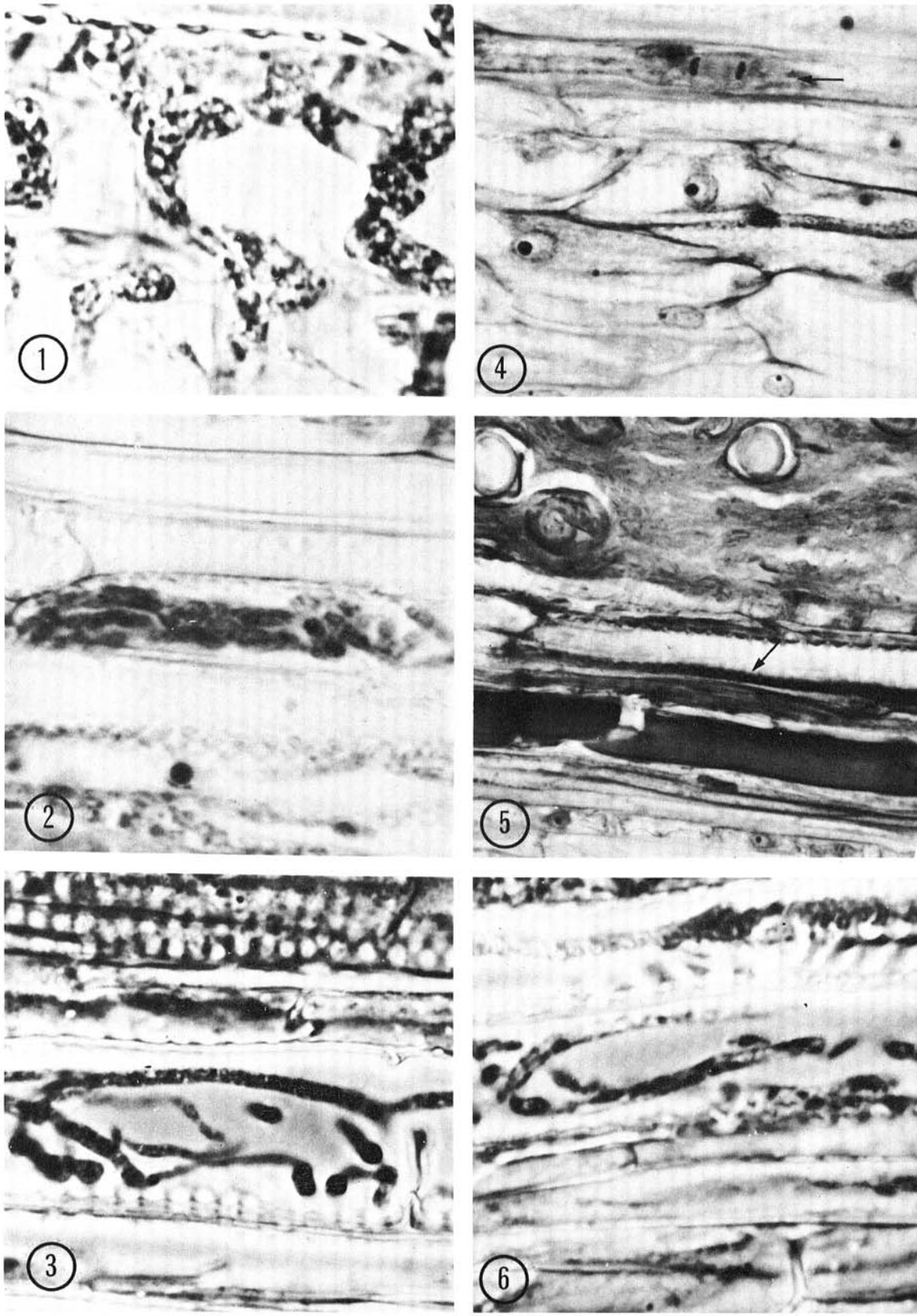
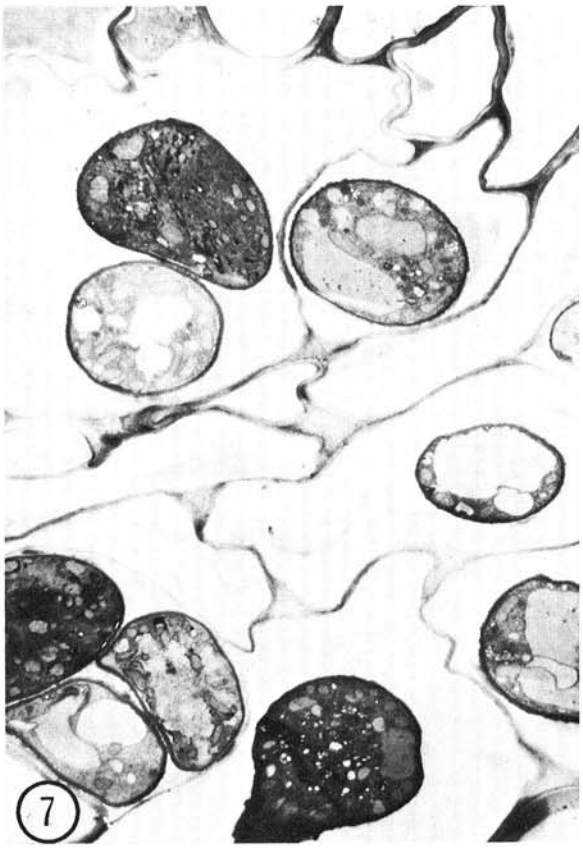
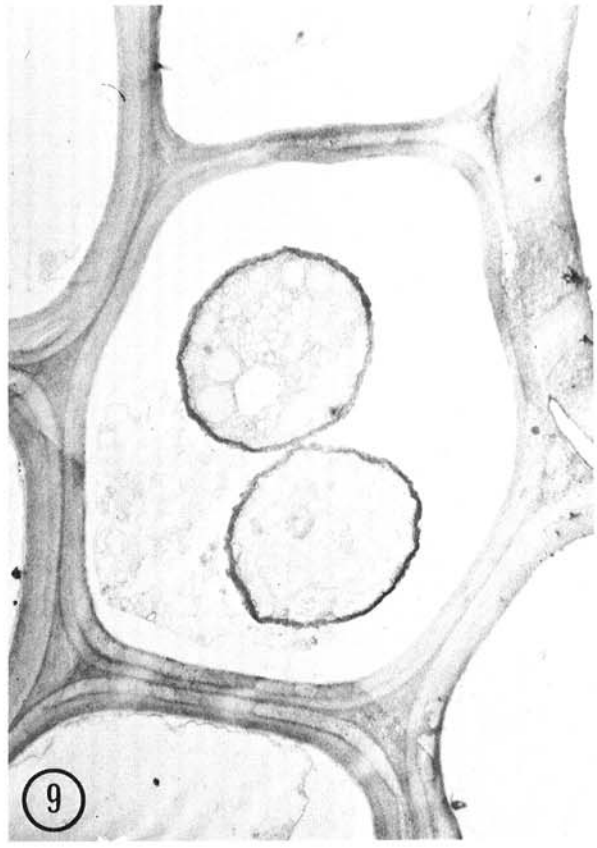
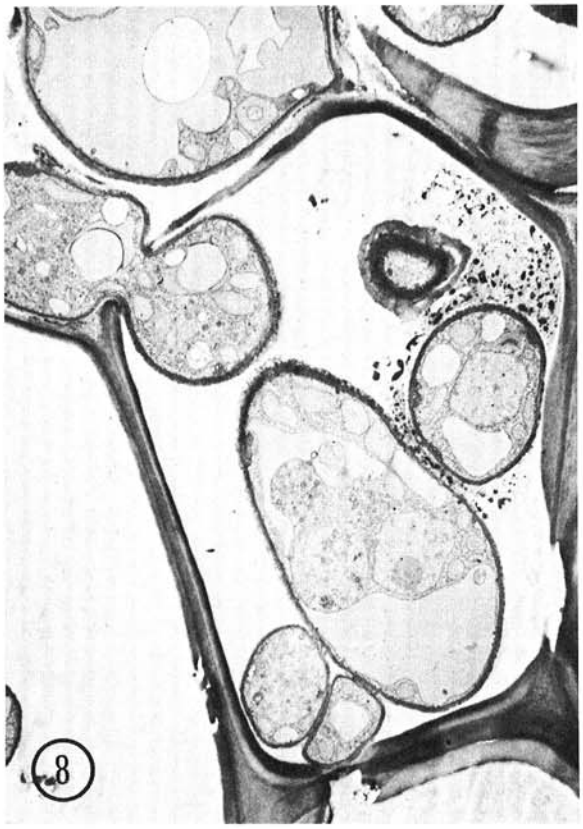


Fig. 1-6. Susceptible **(1-3)** and resistant **(4-6)** soybean hypocotyls three days after inoculation with *Phytophthora megasperma* var. *sojae* (×400, phase-contrast except as noted). **1)** Intercellular hyphae in parenchyma. **2)** Xylem vessel filled with hyphae. **3)** Intracellular hyphae. **4)** Intact and dividing host nuclei (arrow) near site of inoculation (light field). **5)** Dark-staining barrier (arrow) between infected and noninfected host cells. **6)** Xylem vessel containing hyphae.







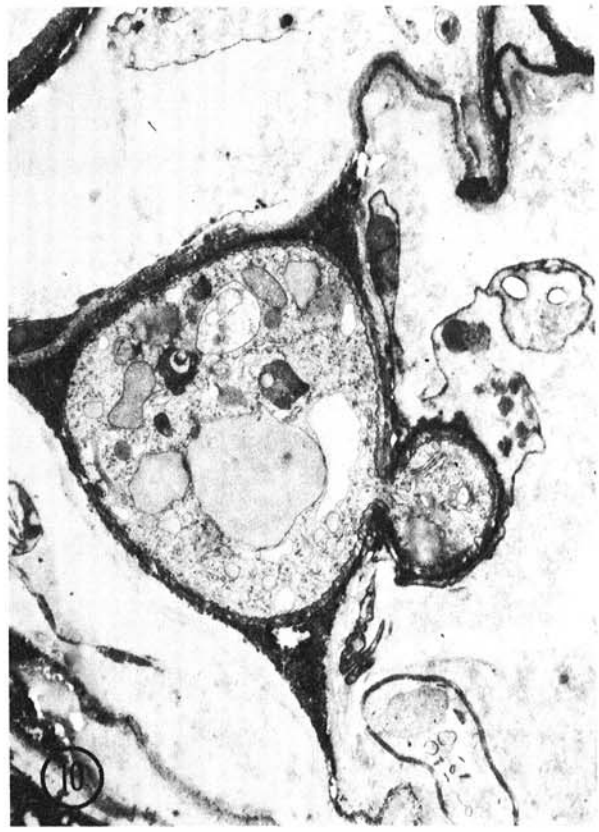
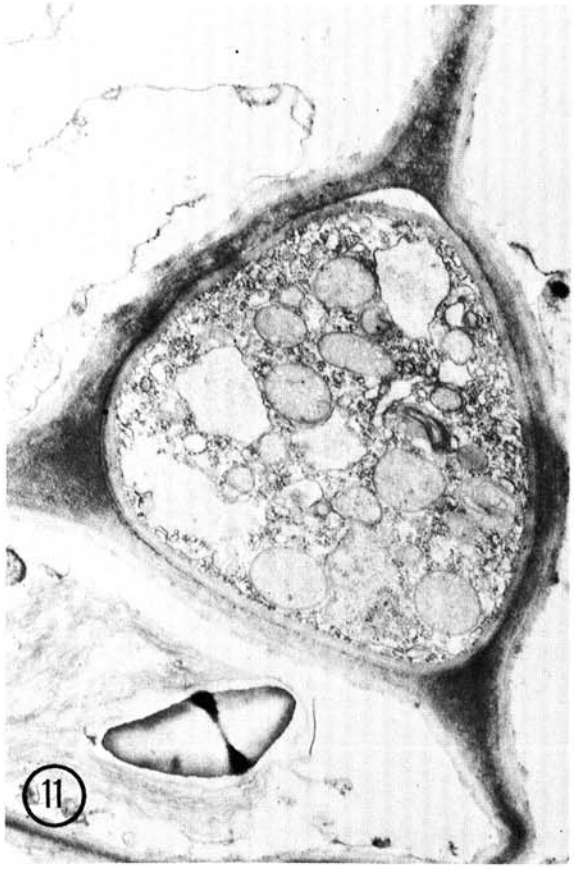


Fig. 7-10. Susceptible soybean hypocotyls three days after inoculation with *Phytophthora megasperma* var. *sojae*. **7)** Intracellular hyphae in disorganized host tissue (×3,220). **8)** Hypha penetrating host cell, and intracellular hyphae (×4,230). **9)** Intracellular hyphae in host cell with secondary wall thickenings (×4,410). **10)** Intercellular hypha with haustorium-like structure penetrating host cell (×6,800).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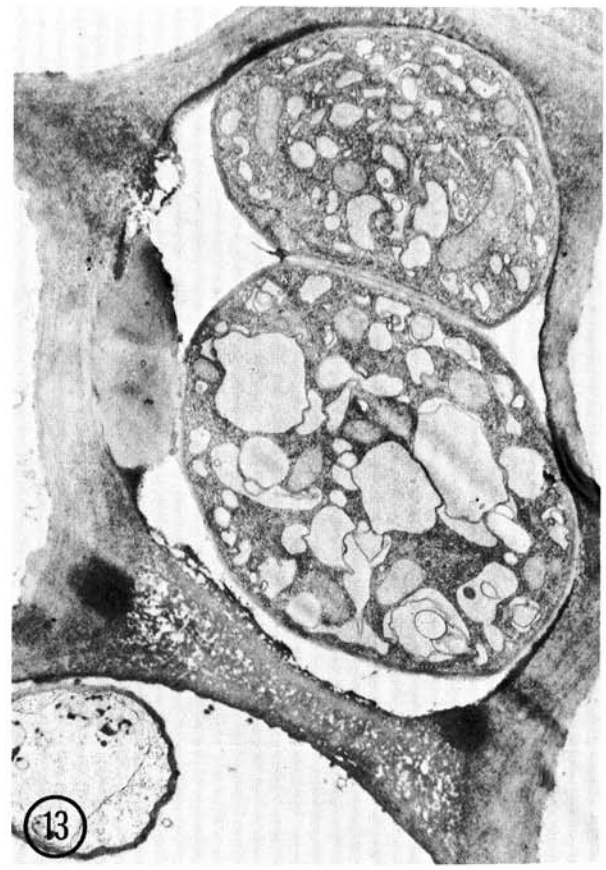
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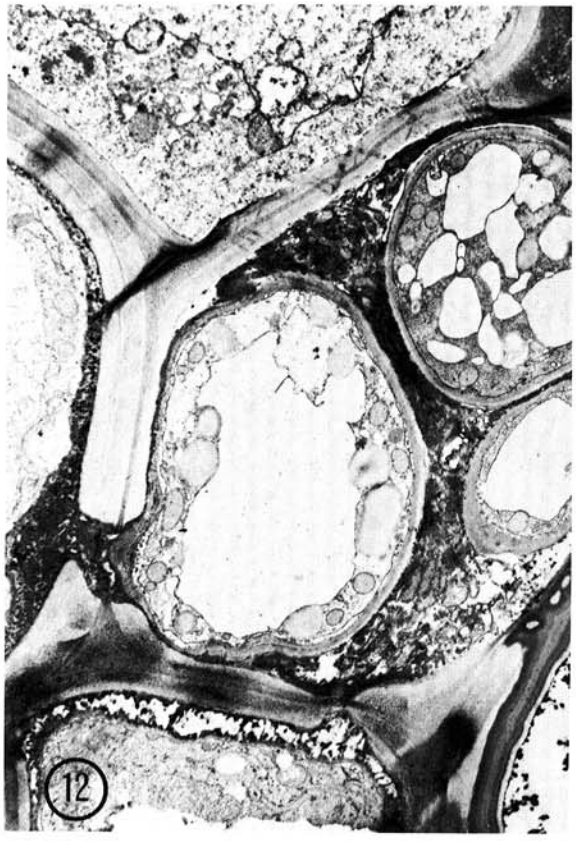
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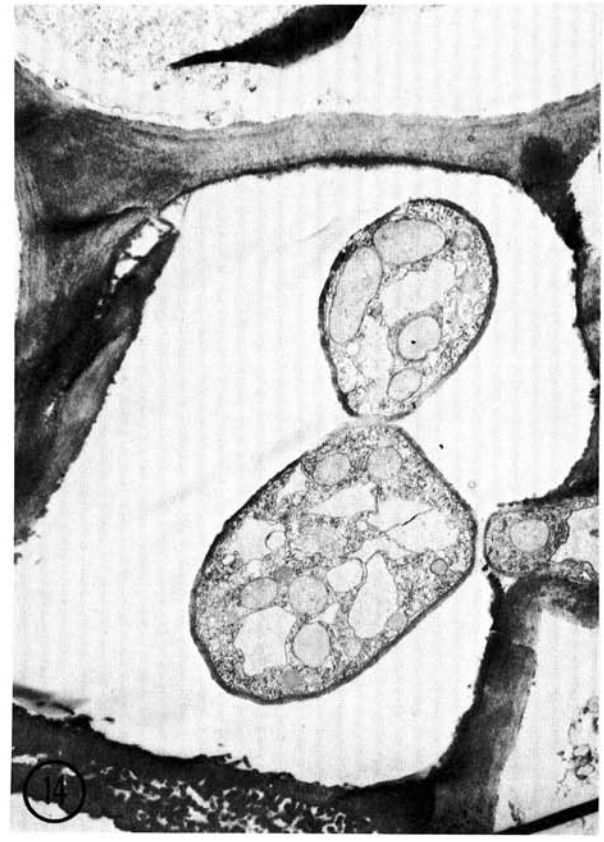
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Fig. 11-14. Resistant soybean hypocotyls three days after inoculation with *Phytophthora megasperma* var. *sojae.* **11)** Intercellular hypha (×13,950). **12)** Infected host cell containing dark-staining cytoplasm moving into two adjacent cells through dissolved areas of the cell wall (×3,075). **13)** Infected host cell with degradation of cell wall (×7,750). **14)** Hypha penetrating host cell already containing hyphae. Note cell wall degradation (×7,350).

13, 14) as in inoculated hypocotyls of H plants. Noninfected host cells adjacent to intercellular *Pms* hyphae often appeared normal. The walls of some infected cells appeared to have undergone general degradation (Figs. 13, 14), while in other cells with thicker secondary walls, only the small portion of the wall touched by the *Pms* hypha appeared to be dissolved (Fig. 12).

In inoculation wounds of H63 hypocotyls, the original inoculum was still present (Fig. 5) and in some cases mycelium had grown out of the wound forming a pad over the surrounding epidermis. Oospores were abundant in this external mycelium, but were not observed to be associated with inoculated H hypocotyls. Colonization of H63 tissues by *Pms* hyphae did not appear sufficient to support such extensive external mycelial growth, but cell contents from inoculation wounds probably provided the necessary nutrients.

Cells of H63 hypocotyls located a short distance from the inoculation wound, contained granular, dark-staining cytoplasm which moved into adjacent cells through the dissolved areas in the cell wall and formed a layer between the membrane and the cytoplasm (Fig. 12). These dark-staining cells (Fig. 5, arrow) seemed to provide a barrier to further *Pms* penetration, because hyphae were never observed to pass from the site of inoculation through these cells and were never found on the other side except in vascular tissues. Similar, dark-staining cells have been reported in other host-pathogen interactions and are considered to be a part of the hypersensitive reaction (11). These cells are probably the origin of the red material earlier described as being associated with production of soybean phytoalexin.

Within one to two cell layers from the inoculum, host nuclei appeared normal (Fig. 5) and many were dividing (Fig. 4, arrow) apparently in response to the inoculation wound. Nuclear division also occurred in cells of wounded H and H63 hypocotyls but not in hypocotyls of inoculated H plants, suggesting that extensive damage to host nuclei occurred rapidly and prevented callus formation.

Vascular tissues of the H63 hypocotyls were colonized, but not as extensively as those of H hypocotyls. Mycelium could be observed in H63 vascular tissues (Fig. 6) some distance from the point of inoculation suggesting that the resistance mechanism was not completely effective. Additional evidence for this was given by Bridge and Klarman (2) who used ultraviolet irradiation to induce high concns of phytoalexin in hypocotyls of susceptible H plants prior to inoculation with *Pms*. Infected, irradiated plants wilted, but did not rot, suggesting that the resistance mechanism had been activated in the epidermal and cortical tissues, but not in the vascular system.

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