Distribution and Ultrastructure of *Peronosclerospora maydis* in Maize Shoot Tips

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ABSTRACT

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The ultrastructure of maize (*Zea mays*) shoot tips, either uninfected or infected with *Peronosclerospora maydis* (the maize downy mildew pathogen), was studied in relation to the systemic infection. Hyphae and haustoria were found in almost every portion of the tip, including the apical meristem, subapical meristem, initial leaf primordium, and leaf primordium. However, hyphae were never observed in the single-cell-thick mantle layer, or the top part of the subapical meristem (25 μm from the top). Degeneration of host cells in the shoot tip of infected plants was seldom encountered and degeneration of proplastids was not observed. All the hyphae and haustoria found in the shoot tip appeared to have been alive and vigorous at fixation, as judged by the ultrastructural appearance of their organelles. The fungus in the apical meristem is considered to extend hyphae and penetrate into the leaf primordium concurrently with shoot tip growth. The ultrastructure of the fungus and host-parasite interface in the shoot tip was similar to that in other downy mildews already reported.

Maize downy mildews caused by *Peronosclerospora maydis* (Racib.) C. G. Shaw, *P. philippinensis* (Weston) C. G. Shaw, *P. sorghi* (Weston and Uppal) C. G. Shaw, and *P. sacchari* (T. Miyake) C. G. Shaw are among the most destructive diseases of maize in Southeast Asia. The disease produces typical systemic sypmtoms. Some histopathological studies in relation to the systemic infection have been conducted by optical microscopy (3,5,10,11). In Indonesia, the disease (which is called Java downy mildew) is caused by *P. maydis*. Harjono (5) studying *P. maydis* stated that the fungus usually did not infect the growing point of the host. However, recently Sudjadi et al (10) reported that *P. maydis* was present in maize shoot tips. It is necessary to define more precisely the relationship between fungal development in the shoot tip and the systemic infection.

The present report deals with investigations on the distribution of the fungal hyphae and the ultrastructure of the fungus and host cells in the shoot tips of maize plants.

MATERIALS AND METHODS

Maize seedlings (*Zea mays* L. 'Golden Cross Bantam,' which is susceptible to the Java downy mildew fungus) at the two- to three-leaf stage (10 days after sowing) were inoculated by spraying them with a conidial suspension of *P. maydis*. Systemic symptoms appeared first on the fifth or sixth leaves, 12–14 days after inoculation. Immediately after the appearance of systemic symptoms, the shoot tip was sampled. Healthy shoot tips were sampled from uninoculated plants grown under the same conditions as the infected plants.

The shoot tips were cut into small pieces, then fixed by using two different methods. In the first method, the pieces were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3–4 hr at 4 C and postfixed in 1% veronal-acetate-buffered osmium tetroxide (pH 7.2) for 3 hr at 4 C. In the second method, the pieces were fixed with 2.5% glutaraldehyde in 0.07 M phosphate buffer (pH 7.0) containing 0.1 M sucrose and postfixed with 1.0% osmium tetroxide in the sucrose buffer described above for 3 hr at 4 C. After fixation, the materials were dehydrated through an acetone series and embedded in epoxy resin. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and observed with a Hitachi-12 electron microscope. Thirteen inoculated, and three uninoculated, meristems were examined.

The identification of each portion of the shoot tip is indicated in Fig. 1 (10). The terminology of the ultrastructure of the host-parasite interface follows that proposed by Kajiwara (8).

RESULTS

Practically none of the host cells of shoot tips infected with the maize downy mildew fungus showed appreciable degenerative changes. Necrotic host cells, which were seldom found, contained degenerated organelles in an electron-dense cytoplasm. The host cells in the tissues of the shoot tip were in close contact with each other and intercellular spaces were rare (Figs. 3–6). These host cells contained characteristic organelles, such as provacuoles and proplastids. Small provacuoles surrounded by a thick tonoplast were found to be scattered in most of the host cells of the apical meristem and peripheral meristem (Figs. 3, 4, 7 and 8). Vacuoles were observed in the cells near the central meristem and leaf primordia (Figs. 6 and 8). A nucleus was present in the central part of the host cytoplasm, which also contained proplastids, mitochondria, lipidlike bodies, and numerous ribosomes (Figs. 7–10). In particular, the host cells of the apical meristem and peripheral meristem contained numerous lipidlike bodies (Figs. 3, 4 and 9). Proplastids and mitochondria in the cells of the shoot tip, except for leaf primordia, showed a close resemblance (Figs. 7, 9, and 11). These two organelles could, however, be distinguished when their ultrastructure was observed in more detail. Mitochondria were spherical or oblate, while proplastids were slightly more slender with more prominent membranes. Futhermore, most of the proplastids contained sparse lamellae and osmiophilic bodies (Fig. 11). Organelles in infected shoot tips were similar in structure and in number to those in healthy ones.

As intercellular hyphae invaded the spaces between the host cells in the shoot tip, the hyphal cell walls were in close contact with the host cell walls, making it difficult to differentiate these hyphae from the host cells. However, the hyphae never contained proplastids and their vacuolar matrix was more electron-dense and contained fibrous material not found in host vacuoles (Figs. 3–8).

Nuclei were present in the hyphae, but were not found in haustoria. In both hyphae and haustoria, there were mitochondria, lomasomes, vacuoles, rough ER, dictyosomes, lipidlike bodies, and numerous ribosomes (Figs. 7–10). Sometimes, the surface of the haustorial neck was covered by a sheath with an electron-lucent

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matrix (Fig. 10). An amorphous, moderately electron-dense layer was present around the haustorium; ie, between the haustorial wall and the host cytoplasm or sheath (Figs. 7, 9 and 10). Among 45 different haustoria seen in sections, the electron-dense layer was observed in 45 (100%), and the haustorial sheath was observed in 23 (51%). Although hyphae and haustoria could not always be easily distinguished from each other, haustorial walls often had two layers, an electron-dense layer and a haustorial sheath.

Ultrathin sections of shoot tips were serially prepared and examined to determine the distribution of intercellular hyphae in the whole shoot tip. The hyphae, which were always found in the central meristem and the leaf primordium, were never detected in the single-cell-thick mantle layer of any of the shoot tips examined. In some of the shoot tips, the hyphae spread over almost all of the shoot tip, including the apical meristem, subapical initials, and peripheral meristem.

The distribution of hyphae was examined in 60 serial ultrathin sections (5 μm thick in total), which were cut longitudinally to include the shoot axis. As shown in Fig. 3, which corresponds to portion "A" in Fig. 2, no hyphae were found in the single-cell-thick mantle layer or in the upper part of subapical initials, whereas they were consistently present in an area more than 25 μm from the tip. Fig. 4, which corresponds to portion "B" in Fig. 2, includes the initial leaf primordium. Hyphae were observed in the tissues of the peripheral meristem, and they were close to, but not in, the single-cell-thick mantle layer. Fig. 5 corresponds to portion "C" in Fig. 2, and involves the initial leaf primordium. Fig. 6 corresponds to portion "D" in Fig. 2, and includes the leaf primordium. Hyphae were frequently observed in these tissues.

The pattern of hyphal distribution in the longitudinal serial sections, including the shoot axis, is illustrated in Fig. 2, where black dots and lines indicate the location of hyphae observed in a shoot tip.

DISCUSSION

One of the characteristic symptoms of downy mildew caused by *Peronosclerospora maydis* and *P. philippinensis* is the systemic chlorosis or the presence of chlorotic stripes on all the leaves which expand after a certain period following infection. Dalmacio et al (3) found that chloroplasts of both healthy and diseased leaves infected with *P. philippinensis* were more or less the same in number and appearance. On the other hand, Weston (11) reported that the chloroplasts of leaf cells infected with *P. philippinensis* were gradually destroyed by the action of the fungus. These results were obtained by optical microscopy. In the present report, electron microscopic observations revealed no degeneration of the proplastids in various portions of the infected shoot tip. The degeneration of chloroplasts observed by Weston might have been caused by the severity of the infection associated with adverse environmental conditions or by observations of old diseased leaves. In the present study, in spite of the use of plants maintained under conditions conducive to severe infection, almost all of the infected shoot tips seemed to be normal, judging from their ultrastructural characteristics. As for the stage of leaf development in which structural abnormality of chloroplasts can be first detected, it will be necessary to investigate the ultrastructure of chloroplasts at various stages of leaf development of the infected plant.

The ultrastructure of the fungus and of the interface between the fungus and host cell in maize shoot tip was found to be identical to that in the mature leaf (K. Takahashi, *unpublished*). The ultrastructure also was similar to that of *Peronospora manshurica* (9), *P. parasitica* (2), *P. destructor* (7), *P. brassicae* (7), *P. spinaciae* (7) and *Pseudoperonospora cubensis* (6). The fungus in the shoot tip in particular, seemed to be alive and vigorous as judged from the ultrastructure of the organelles in hyphae.

On the basis of optical microscopic observations on sections of maize shoot tips infected with the downy mildew fungus, it could be demonstrated that the hyphae of *P. maydis* were observed in the shoot tip including the central meristem and the leaf primordia, but not in the apical meristem (10). In the present report, electron microscopic observations confirmed the presence of *P. maydis* in various portions of the shoot tip, not only in the central meristem and the leaf primordia (including the initial primordium), but also in the apical meristem. That hyphae of *P. maydis* could not be detected in the apical meristem of the maize shoot tip by optical microscopy in a previous report (10), may be ascribed to insufficient contrast due to the similar densities of host and fungal cytoplasm in the apical meristem and the close contact between the slender hyphae and host cell walls.

The apical meristem is at the origin of various portions of the shoot tip, and the initial leaf primordium is formed by cell division in the peripheral meristem on the side of the shoot tip (4). The initial leaf primordium develops into the leaf primordium, and then into the mature leaf (1). It is thus highly significant that the fungus was

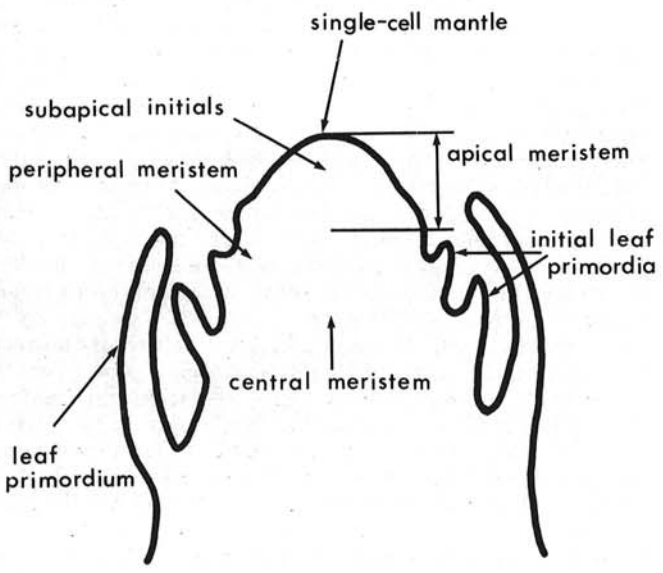


Fig. 1. Diagram of a maize shoot tip.

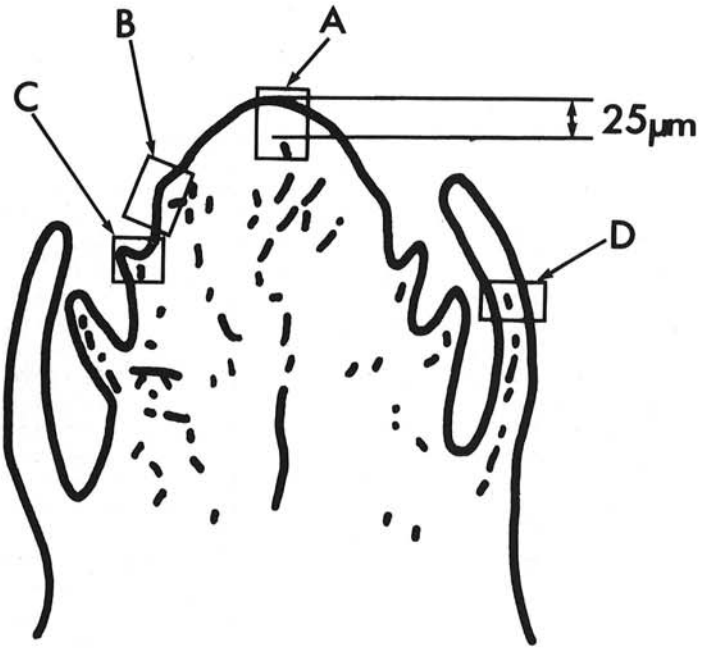
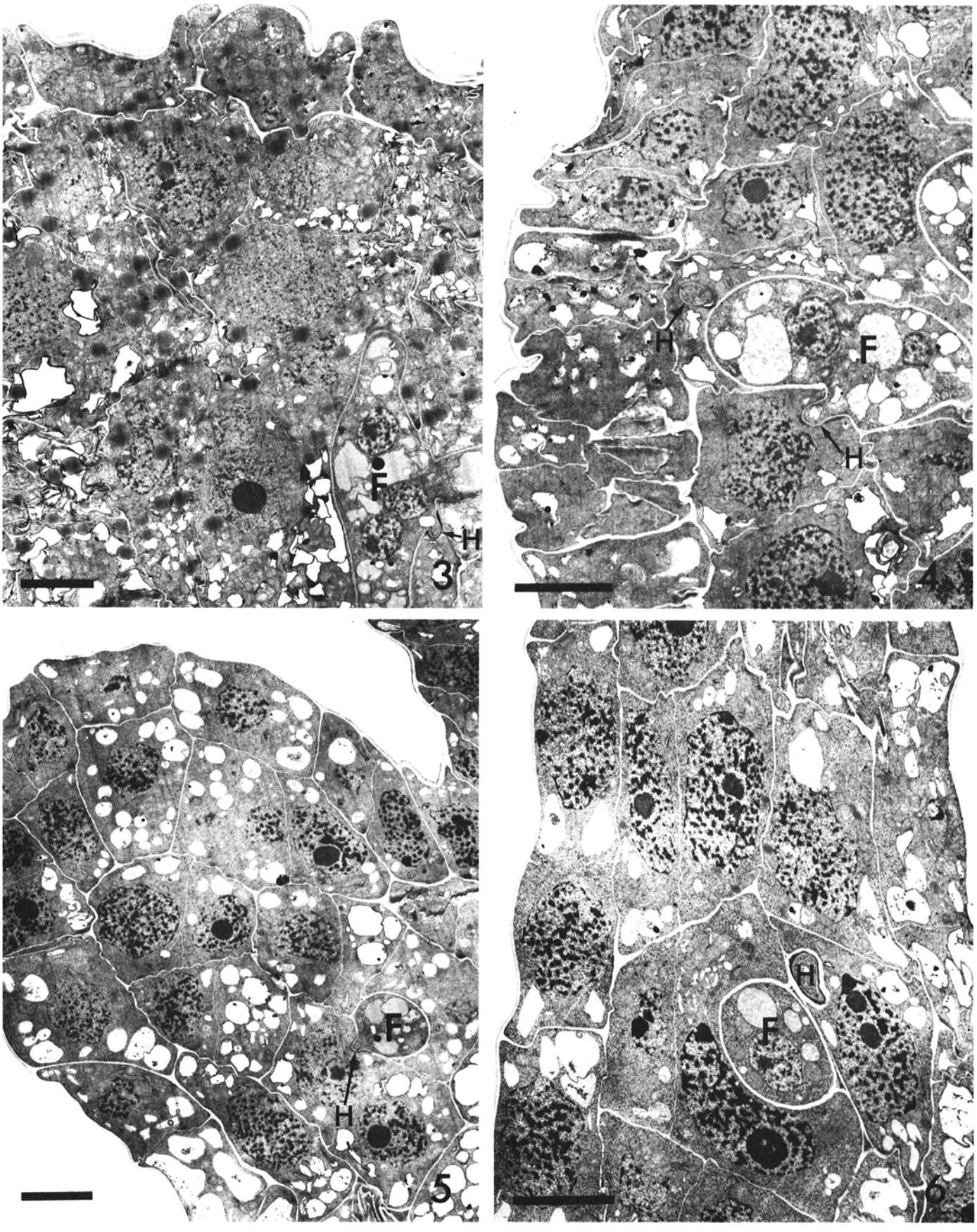
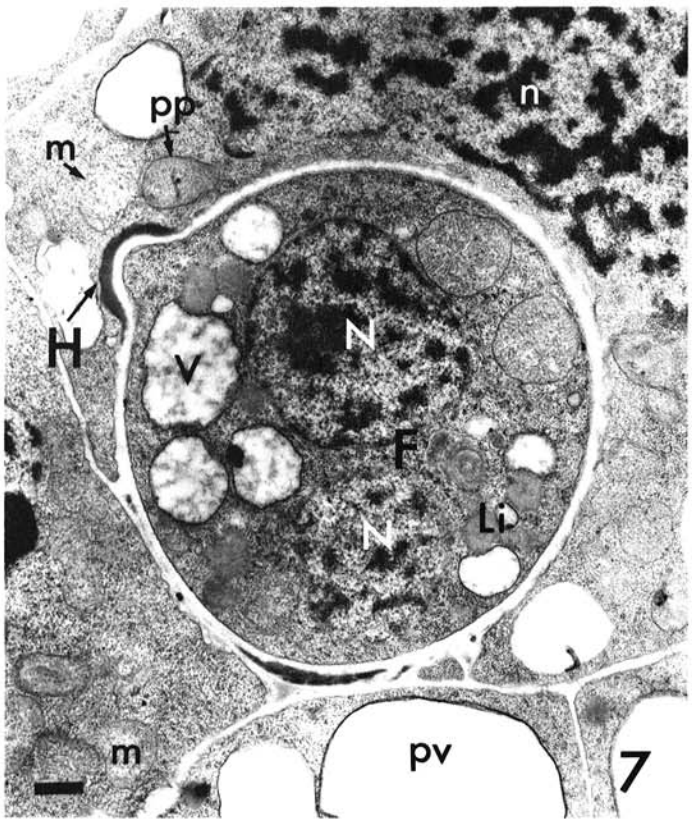
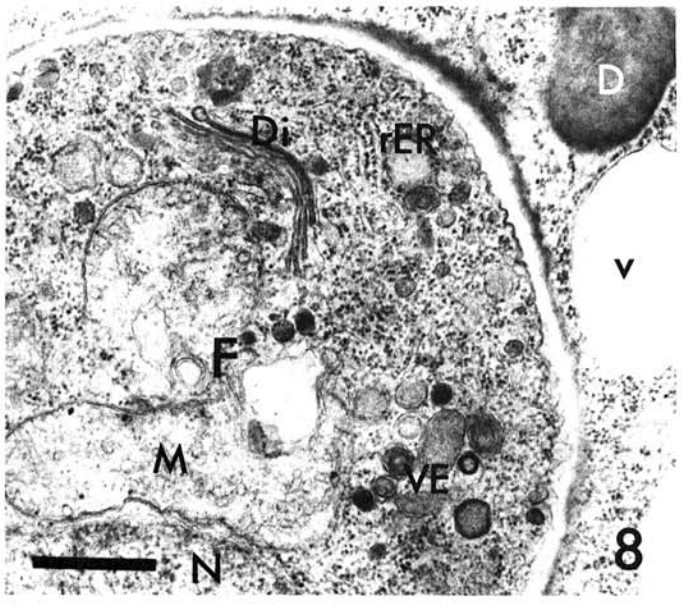


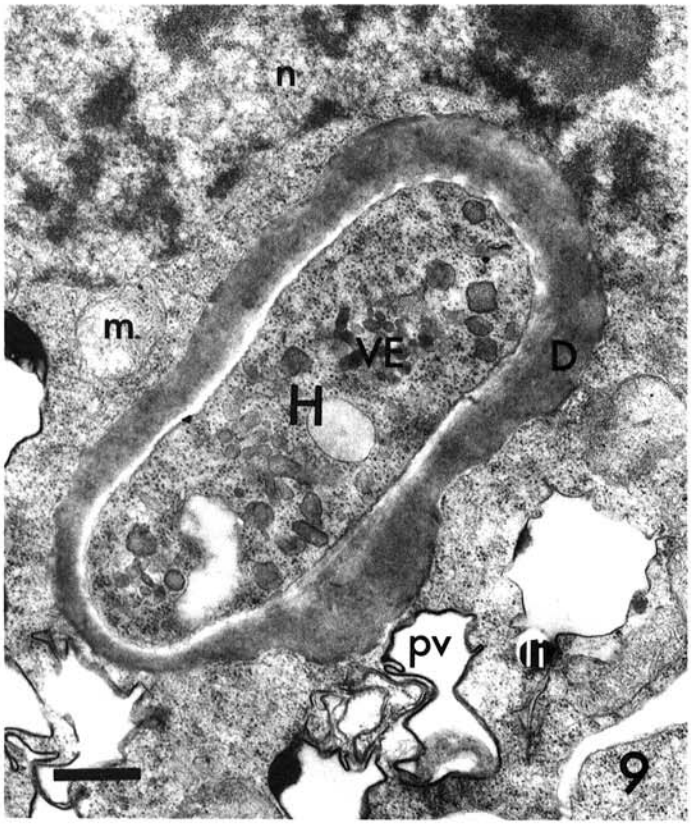
Fig. 2. Distribution of hyphae in a maize shoot tip infected by *Peronosclerospora maydis*. The location of hyphae indicated by black dots and lines was examined in 60 serial ultrathin sections (5 μm thick), which were cut longitudinally to include the shoot axis. "A" portion includes the single-cell-thick mantle layer and the upper part of subapical initials. "B" and "C" portions include the initial leaf primordium. "D" portion includes the leaf primordium.

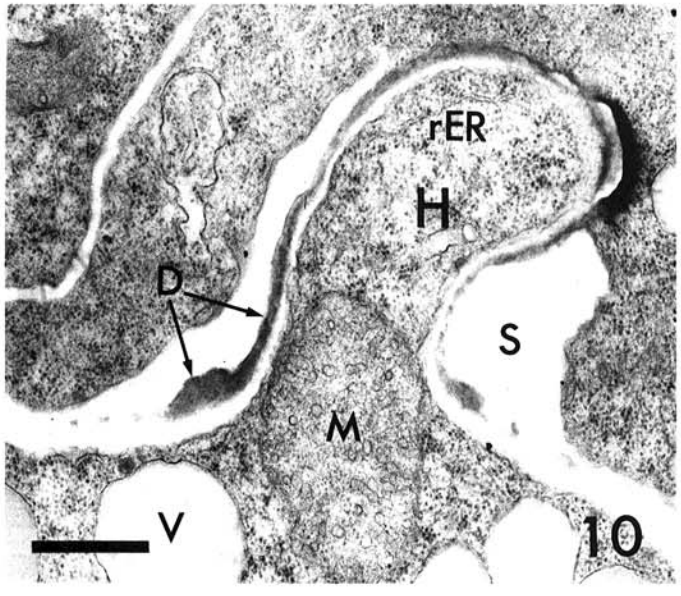


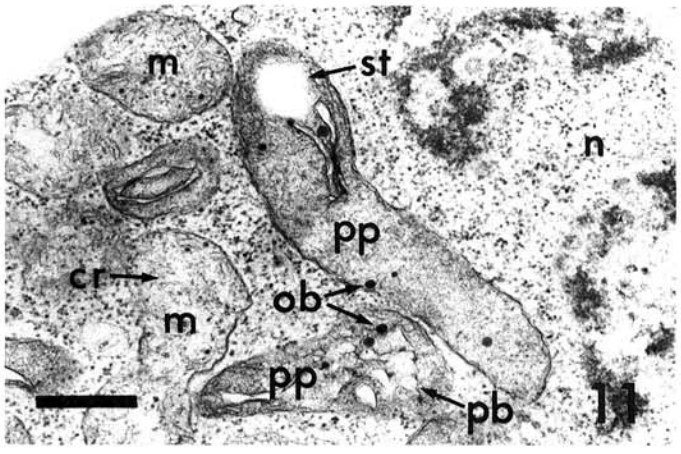
Figs. 3–6. Maize shoot tip infected by *Peronosclerospora maydis*. **3,** Intercellular hypha (F) and haustorium (H) in the apical meristem, "A" portion in Fig. 2. **4,** Intercellular hypha (F) and haustoria (H) in the initial leaf primordium, "B" portion in Fig. 2. **5,** Intercellular hypha (F) and haustorium (H) in the initial leaf primordium, "C" portion in Fig. 2. **6,** Intercellular hypha (F) and haustorium (H) in the leaf primordium, "D" portion in Fig. 2. Scale bar represents 5μm.











Figs. 7–11. Host-parasite interfaces of maize shoot tip infected with *Peronosclerospora maydis*. **7,** Intercellular hypha (F), and young haustorium (H). An electron-dense layer surrounds the haustorial cell wall. **8,** Intercellular hypha (F) including several cytoplasmic organelles. Note numerous vesicles (VE). **9,** Tip portion of a haustorium (H) being formed in a host cell of the apical meristem. Note numerous vesicles (VE) in the haustorial cytoplasm. **10,** Tangential section of a haustorial initial (H) in a host cell. Note the sheath (S) around the haustorial neck. **11,** Proplastids and mitochondria in the host cell of the shoot tip. cr = host mitochondrial cristae; D = electron-dense layer; Di = fungal dictyosome; Li = fungal lipidlike body; li = host lipidlike body; M = fungal mitochondrion; m = host mitochondrion; N = hyphal nucleus; n = host nucleus; ob = osmiophlic body; pb = prolamellar body; pp = proplastid; pv = provacuole; rER = fungal rough surface endoplasmic reticulum; st = starch granule; V = fungal vacuole; v = host vacuole. Scale bar represents 0.5 μm.

commonly detected in the apical meristem and initial leaf primordium as well as frequently observed in the leaf primordium and central meristem. The hyphae in the apical meristem and the central meristem are considered to invade the newly developing initial leaf primordium. The hyphae may continue to grow in the leaf tissue throughout the development of the leaf, successively colonizing the initial leaf primordium, the leaf primordium, and the young leaf. Thus, it is thought that a plant infected with *P. maydis* in the early stage of growth will display systemic symptoms manifested by chlorotic stripe involving the whole leaf. On the other hand, in a leaf which is already developed or expanded at the time of the colonization of *P. maydis* in the maize shoot tip, the hyphae seem to be unable to extend into the old portion of the leaf. Therefore, the so-called "half-diseased leaf" may be observed in the initial stage of development of the systemic symptoms.

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