Cellular interaction of the smut fungus *Ustacystis waldsteiniae*[1]

Robert Bauer, Kurt Mendgen, and Franz Oberwinkler

Abstract: The cellular interaction between the smut fungus *Ustacystis waldsteiniae* and its host *Waldsteinia geoides* was analyzed by serial-section electron microscopy using chemically fixed and high-pressure frozen – freeze-substituted samples. After penetration, each haustorium extends a short distance into the host cell where it often forms up to three short lobes. The haustorium is wholly ensheathed by a prominent matrix. The matrix is a complex structure, differing significantly from that known of other fungal plant parasites: it is filled with amorphous, electron-opaque material in which membrane-bounded, coralloid vesicles are embedded. During the contact phase of the hypha with the host cell wall, vesicles with electron-opaque contents accumulate in the contact area of the hypha where they appear to fuse with the fungal plasma membrane and extrude their contents. Subsequently, the host cell wall increases in electron opacity and matrix material becomes deposited between host plasma membrane and host cell wall exactly at the ends of the altered areas in the host cell wall. The coralloid vesicles within the matrix, however, are of host origin: exocytosis of Golgi products into the matrix results in the formation of coralloid vesicular buds in the host plasma membrane. Subsequently, the buds seem to detach from the host plasma membrane to flow as coralloid vesicles into the matrix. Matrix development continues during penetration and after penetration at the haustorial tips. After host wall penetration, the fungal cell wall comes in contact with the matrix. The fungal component of the matrix may play a key role in the inducement of these transfer cell-like compartments in host cells responding to infection.

Key words: freeze substitution, haustoria, high-pressure freezing, host–parasite interaction, smut fungi, *Ustacystis waldsteiniae*.

Résumé : L'interaction cellulaire entre le champignon du charbon *Ustacystis waldsteiniae* et son hôte, le *Waldsteinia geoides* a été analysée en microscopie électronique sur sections sériées et en utilisant des échantillons fixés chimiquement ou cryo-substitués sous haute pression. Après la pénétration, l'haustérie s'étend sur une courte distance dans la cellule de l'hôte où il forme souvent jusqu'à trois lobes. L'haustérie est totalement enveloppée dans une matrice bien visible. La matrice est une structure complexe, différent significativement de celle qu'on connait chez d'autres parasites fongiques des plantes; il est rempli d'un matériel amorphe opaque aux électrons dans lequel baignent des vésicules coralloïdes enveloppées dans une membrane. Au cours de la phase de contact des hyphes avec la paroi de la cellule hôte, les vésicules avec des contenus opaques aux électrons s'accumulent dans la région de contact des hyphes, où ils semblent se fusionner avec la plasmalemme fongique et expulser leurs contenus. Subséquemment, l'opacité aux électrons de la paroi de la cellule hôte augmente et le matériel matriciel se retrouve entre la plasmalemme de l'hôte et la paroi cellulaire de l'hôte, exactement aux extrémités des surfaces altérées de la paroi cellulaire de l'hôte. Les vésicules coralloïdes de la matrice, appartiennent cependant à l'hôte : l'exocytose de produits de l'appareil de Golgi dans la matrice conduit à la formation de bourgeons sur les vésicules coralloïdes, dans la plasmalemme de l'hôte. Subséquemment les bourgeons semblent se détacher de la plasmalemme de l'hôte pour s'épancher sous forme de vésicules coralloïdes dans la matrice. Le développement de la matrice se poursuit au cours de la pénétration des bouts des haustéries. Après la pénétration de la paroi cellulaire de l'hôte, la paroi cellulaire fongique vient en contact avec la matrice. La composante fongique de la matrice pourrait jouer un rôle clé dans le déclenchement de la formation de ces compartiments de transfert ressemblant à des cellules, chez les cellules hôtes, en réaction à l'infection.

Mots clés : cryo-substitution, haustéries, congélation sous haute pression, interaction hôte–parasite, champignons du charbon, *Ustacystis waldsteiniae*.
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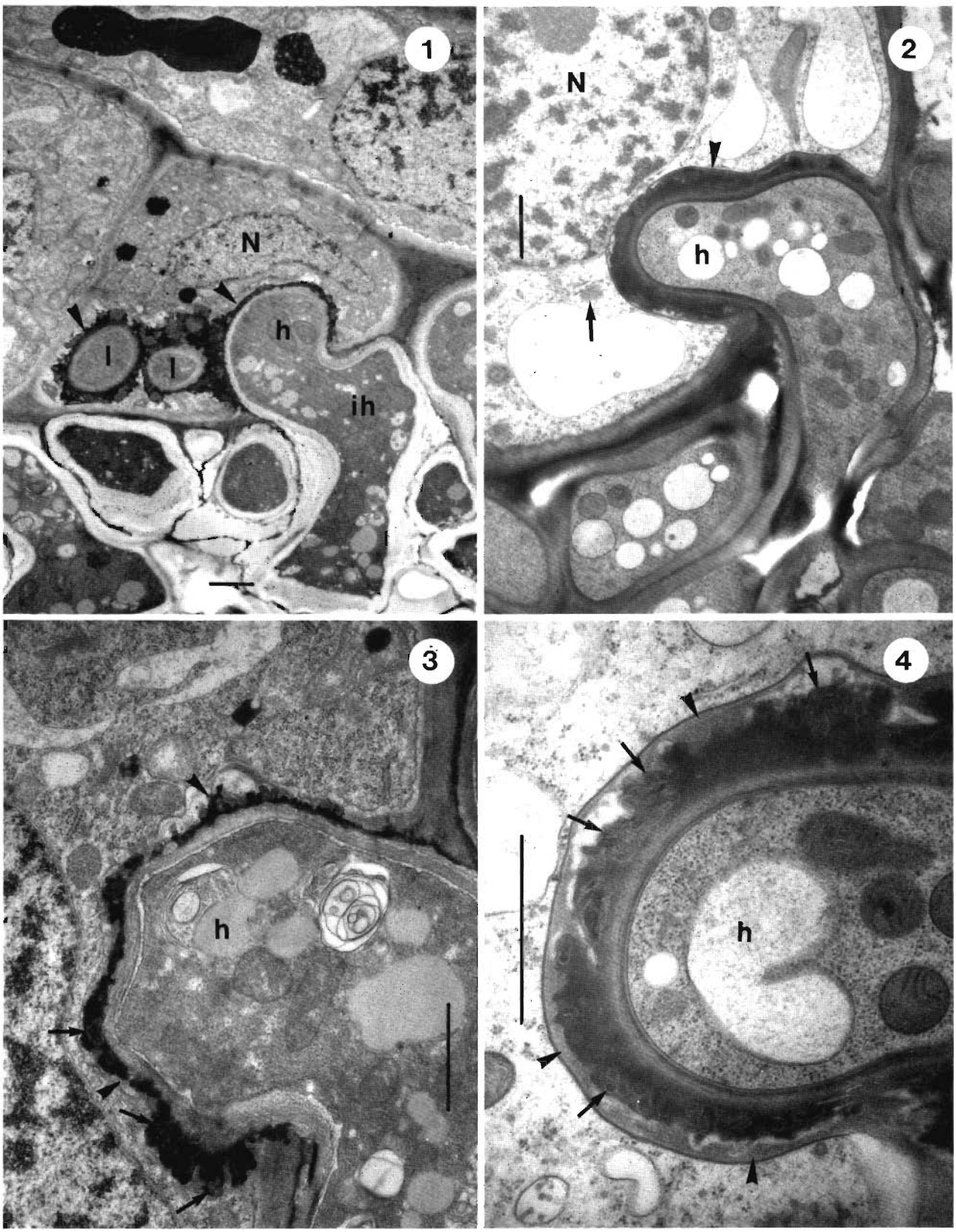
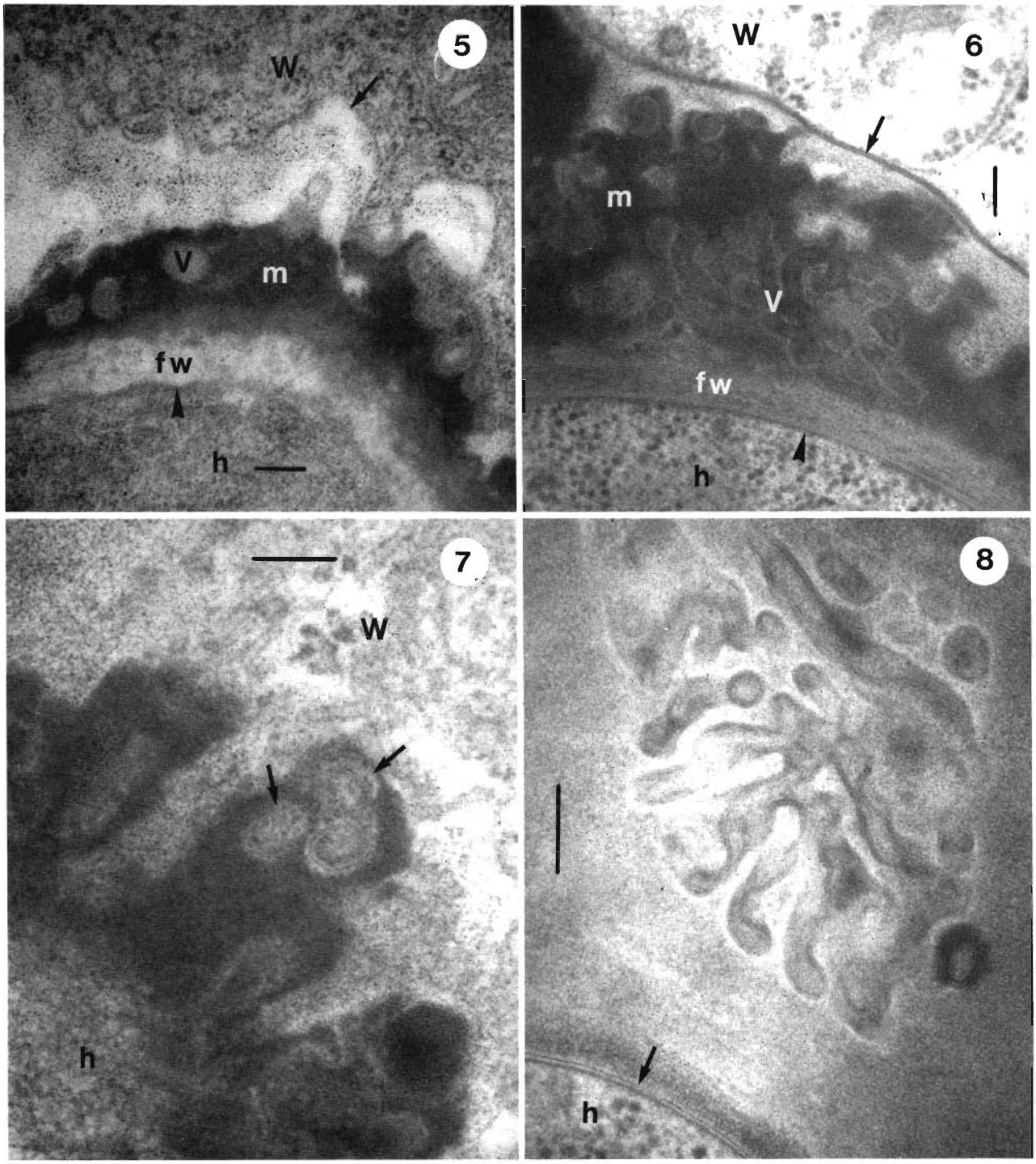
Figs. 1–4. Haustoria prepared by conventional fixation (Figs. 1 and 3) or freeze substitution (Figs. 2, 4). Scale bars = 1 μm. Fig. 1. Section showing a conventionally fixed haustorium (*h*) with two sectioned lobes (*l*). The haustorium is continuous with its intercellular hypha (*ih*). The electron-opaque matrix surrounding the haustorium is recognizable (arrowheads). The host nucleus is visible at N. Fig. 2. Presumed young haustorium (*h*) after freeze substitution illustrated to show the well-preserved cytoplasm of both partners. The membranes are smooth. Note that the matrix (arrowhead) surrounding the haustorium is



Figs. 5–8. Interfaces between haustoria (*h*) of *Ustacystis waldsteiniae* and host cells of *Waldsteinia geoides* (W in Figs. 5–7) prepared by conventional fixation (Figs. 5, 7) or freeze substitution (Figs. 6, 8). Scale bars = 0.1 μm. Figs. 5 and 6. Conventionally fixed interface (Fig. 5) and freeze-substituted interface (Fig. 6) with fungal plasma membrane (arrowheads), fungal cell wall (*fw*), matrix (*m*), matrix vesicles (V), and extrahaustorial membrane (arrows) for comparison. The matrix is thicker and electron lighter, the enclosed vesicles appear clearer, and the extrahaustorial membrane is much smoother after freeze substitution (Fig. 6) than after conventional fixation (Fig. 5). Note the irregularly folded extrahaustorial membrane in Fig. 5. Fig. 7. Typical appearance of the conventionally fixed matrix vesicles (arrows). The contours of the matrix vesicles are recognizable, but their membranes appear unclear. Fig. 8. Section showing a typical highly branched matrix vesicle after freeze substitution. The tripartite nature of the vesicle membrane appears clear. Note the unequal layering of the fungal plasma membrane (arrowhead).



rhizomes are infected. Sori of the smut fungus were formed, however, only on leaves produced in the spring, whereas the leaves produced in the summer and autumn from the same rhizomes did not show any infection. The fungus first appeared in the centre of very young main veins near the leaf margin and caused a significant hypertrophy and an anatomical change of the infected veins; typical vascular elements were not differentiated. Hyphae grew intercellularly in the centre of the altered veins and filled the intercellular spaces in that region nearly completely (Figs. 1, 2). Aseptate haustoria (for definition see Bushnell 1972) arose from intercellular hyphal cells that contacted host cells (Figs. 1, 2). Although they were not constricted at the penetration point and specialized structures, such as haustorial mother cells, osmiophilic rings, collars, and papillae were absent (Figs. 1–3), they had a characteristic morphology distinct from intercellular hyphal cells: each haustorium extended a short distance into the host cell where it often formed up to three aseptate short lobes (Fig. 1). It always terminated in the host cell and was surrounded by an extrahaustorial membrane that was continuous with the host plasma membrane (Figs. 2 and 3). More than one haustorium sometimes arose from the same hyphal cell and sometimes these entered different host cells. Several haustoria from different hyphal cells were also observed in a single host cell (not illustrated).

By both fixation techniques the general haustorial architecture was recognizable. In freeze-substituted material, however, haustoria had a more regular appearance and were in most cases more distinct than after conventional fixation (compare Fig. 1 with Fig. 2 and Fig. 3 with Fig. 4). After freeze substitution, the substructure of the host plasma – extrahaustorial membrane appeared different from that of the fungal plasma membrane (compare Fig. 8 with Fig. 12): the two electron-opaque layers of the host plasma – extrahaustorial membrane were more or less equal in thickness (Fig. 12). The thickness of each of these layers was comparable with that of the protoplasmic electron-opaque layer of the fungal plasma membrane. The exoplasmic layer of the fungal plasma membrane, however, appeared thinner (Fig. 8).

The most interesting feature of the interaction of *Ustacystis waldsteiniae* was the prominent and distinct matrix that separated the extrahaustorial membrane from the haustorial cell wall. The matrix was thicker than the haustorial cell wall and exhibited a smooth profile after freeze substitution (Figs. 2, 4, 6) but not after conventional fixation (Figs. 1, 3, 5). It was composed of amorphous electron-opaque material in which membrane-bounded vesicles were embedded (Figs. 4–7). Electron-transparent lacunae occasionally were distributed throughout the matrix (Figs. 4, 6). The matrix appeared much thicker after freeze substitution than after conventional fixation (compare Fig. 1 with Fig. 2, Fig. 3 with Fig. 4, and Fig. 5 with Fig. 6). In addition, in sections of the same thickness the conventionally fixed matrix was usually of greater electron opacity than the freeze-substituted matrix (compare Fig. 3 with Fig. 4, and Fig. 5 with Fig. 6).

After freeze substitution, the extrahaustorial membrane was generally smooth in the regions of direct contact with the electron-opaque matrix component (Figs. 10, 11), and ladder-like connection structures existed occasionally between both components (Figs. 11, 38, 41). In areas of direct contact with the electron-transparent lacunae, however, the extrahaustorial membrane usually showed undulations extending into the cytoplasm (Figs. 10 and 11, also visible in Figs. 4 and 42). Cisternae of endoplasmic reticulum, mitochondria, and Golgi bodies were frequently present in the host cytoplasm next to the haustoria (Figs. 2, 9, 12). Golgi bodies were normally orientated to the matrix profile with their peripheral regions (Figs. 2, 9, 12). Cisternae of endoplasmic reticulum were frequently found in intimate contact with the extrahaustorial membrane extending from the cytoplasm to the membrane and then following the membrane contours closely (Figs. 12–14). In addition, at the contact areas they were devoid of ribosomes, and small connection filaments of poor contrast were often arranged in a more or less regular spacing (Figs. 12–14). In few cases the matrix was encased partly at the haustorial flanks or completely by appositional material (Figs. 4, 9). Material resembled host cell wall and was continuous with it (Fig. 9). In some of these cases the matrix appeared thinner and only a few matrix vesicles were enclosed.

The tripartite nature of the membranes of the enclosed matrix vesicles was clearly recognizable after freeze substitution (Fig. 8) but not after conventional fixation (Fig. 7). As indicated by serial sections, in advanced haustorial stages the matrix vesicles were separated from both the fungus and the host (Figs. 4, 6, 9, serial sections not illustrated). Though distributed throughout the matrix (Fig. 4), their numbers varied from haustorium to haustorium. Matrix vesicles were highly branched with many lobes that gave the vesicles a coralloid appearance (Fig. 8). A very thin electron-transparent line followed the contours of vesicle membranes closely (Figs. 6–8). In older interaction stages the matrix vesicles were frequently collapsed (not illustrated).

Fungal development

In the initial stages of interaction, the fungal wall was very thin at the contact area with the host cell wall, and difficult to distinguish from the host cell wall after freeze substitution (Figs. 15–18) and conventional fixation (Figs. 25–28). During the contact phase of the hypha with the host cell wall the matrix began to develop and appeared at the host side between host cell wall and host plasma membrane (Figs. 15, 18). Development continued during the penetration process (Figs. 16, 17) and after penetration at the haustorial tips during haustorial growth (Figs. 19, 20). The penetration hypha thus first came in contact with the matrix after penetration. It never had direct contact with the host plasma – extrahaustorial membrane.

Material corresponding to the electron-opaque matrix material was not found in the cytoplasm of host cells in contact with penetration hyphae. During development of the matrix, two different types of vesicle complexes were located in the hyphal tips (Figs. 15, 17, 18). The first type was observed only in the regions of interaction and consisted of vesicles and straight tubules with electron-opaque contents that corresponded to the densely stained material of the matrix (Figs. 15, 21–23). Tubules showed usually one or several margin bulgings and buds at one or both ends, indicating formation of the vesicles (Figs. 21, 22). Compared with the appearance after freeze substitution, after conventional fixation the vesicle complex was typically altered: like the

Figs. 9–14. Host characteristics around haustoria prepared by freeze substitution. Fig. 9. The matrix surrounding the haustorium (*h*) is encased by appositional material (arrows) which is continuous with the host cell wall (arrowhead). Golgi bodies, cisternae of endoplasmic reticulum, and mitochondria are neighboured. Note the unusual orientation of the Golgi body to the haustorium. Scale bar = 0.5 μm. Figs. 10 and 11. At the points of direct contact to the electron-opaque matrix material (*m*) the extrahaustorial membrane appears smooth (arrowheads), whereas at the other areas it has an undulating topography. In Fig. 11 ladder-like connections extend between the matrix and the extrahaustorial membrane (arrows). Note the coated membrane in Fig. 10 at arrow. Scale bar = 0.5 μm in Fig. 10 and 0.1 μm in Fig. 11. Fig. 12. Mitochondrion (M), Golgi body (D), cisterna of endoplasmic reticulum (ER), and coated vesicle (large arrowhead) in close proximity to the extrahaustorial membrane (arrow). The cisterna extends from the cytoplasm to the extrahaustorial membrane and then follows the contour of the membrane closely. At the contact area with the extrahaustorial membrane it is devoid of ribosomes and fine lines exist between both (small arrowheads). Note that the Golgi cisternae are oriented with their peripheral regions to the matrix. Note also the equal layering of extrahaustorial membrane. Scale bar = 0.1 μm. Fig. 13. Cisterna of endoplasmic reticulum (ER) with numerous ribosomes extends from the cytoplasm to the extrahaustorial membrane (arrow). Ribosomes are absent at the contact area. Scale bar = 0.1 μm. Fig. 14. Fine filaments (arrowheads) are seen between the extrahaustorial membrane (arrow) and the intimately associated cisterna of endoplasmic reticulum (ER). Ribosomes are absent in this area. Scale bar = 0.1 μm.

Figs. 15–20. Contact and penetration phase prepared by freeze substitution. Scale bars = 0.5 μm. Fig. 15. Intercellular hypha (*ih*) contacting host cell wall (HW). Matrix material (*m*) is deposited between host cell wall (HW) and host plasma membrane. The first vesicular buds are evident (arrow). Numerous vesicles containing electron-opaque contents (vesicle complex 1) accumulate at the hyphal tip. Note that the fungal cell wall (*fw*) is difficult to distinguish from the host cell wall (HW). Fig. 16. Intercellular hypha (*ih*) lodged in the host cell wall (HW). A cisternal bud of a Golgi body (arrow) is seen in intimate contact with the matrix (*m*). Fig. 17. Intercellular hypha (*ih*) penetrating host cell wall (HW). Behind the area of vesicle complex 1 a second vesicle complex (type 2) is visible within the penetration dome. Note the young matrix (*m*). Fig. 18. Intercellular hypha (*ih*) contacting host cell wall (HW). Vesicle complex 2 is positioned next to the hyphal tip. The fungal and host cell wall are more or less indistinguishable from each other. Note the young matrix (*m*) and the vesicular bud in the host plasma membrane (arrow). Figs. 19 and 20. Interaction stages after penetration. Each hypha (*ih*) contacts the matrix and the fungal cell wall appears clearer than in penetration stages.

electron-opaque matrix material itself, the densely stained contents of the vesicles and tubules were more concentrated and were separated from the vesicle–tubule membrane by an electron-transparent, unstructured sheath (Fig. 24). The vesicles were seen in close contact with the fungal plasma membrane at the hyphal tip, suggesting exocytosis (Figs. 15, 17, 21). In addition, fusion and secretion profiles were present (Figs. 21, 28).

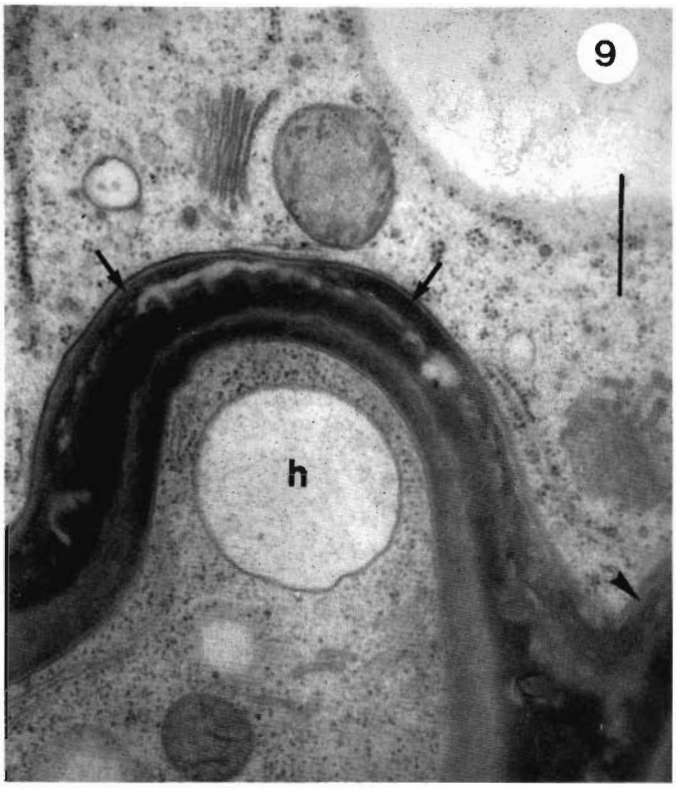
Deposition of electron-opaque material between host plasma membrane and host cell wall was associated with a change in the structure of the host cell wall in the region of interaction. This change appeared much more distinct in conventionally fixed material (Figs. 25–28) than in freeze-substituted material (Figs. 29, 30), especially in thick host cell walls (Figs. 25–28). The change was visible as a granular increase in the electron opacity of the host cell wall starting from the hyphal tip (Fig. 25) and extending through the host cell wall towards the host plasma membrane (Figs. 26, 27). Subsequently, electron-opaque granules became deposited between host cell wall and host plasma membrane exactly at the ends of the altered areas in the host cell wall (Figs. 26, 27). The host plasma membrane was intimately attached to the electron opaque granules, whereas it was undulated between them (Figs. 26, 27). Deposition of electron-opaque material then continued (Fig. 28) to form the matrix. In all stages observed the young matrix was not encased by a secondary wall layer of the host of collar material.

The second vesicle complex seen in the tip of the penetration hypha (Figs. 17, 18) also was found in the tips of intercellular hyphae that were not in contact with host cells. It consisted of electron-transparent, variously shaped segments that were irregular, ringed, and curved with extensions or agglomerations (Figs. 31–34). The two different vesicle complexes were often found adjacent to each other but were never in direct contact (Fig. 17). In addition, in the apical penetration dome either the first vesicle complex was centrally positioned and the other type was neighboured or vice versa (Figs. 17, 18).

Host response

Host response to penetration and haustorial growth followed a defined pattern. Beginning with the contact and penetration phase and continuing during haustorial growth at the haustorial tips, vesicular buds arose from the host plasma membrane around the matrix extending into the matrix (Figs. 15, 18, 40, 41). There seemed to be a regular spacing of the buds. Initially they were more or less globular in shape, and later they elongated and formed many coralloid branches (Figs. 40–45). The membranes of the vesicular buds were continuous with the host plasma – extrahaustorial membrane (Figs. 40–45). The inner space was organelle free, including ribosomes, and was filled with material of greater electron opacity than the adjacent cytoplasm areas (Figs. 40–45). Bud openings were sometimes occluded by cisternae of endoplasmic reticulum (Fig. 45). Additionally, cisternae were seen extending into the inner space of the buds (Fig. 42). A fine electron-transparent line normally followed closely the contours of these buds (Figs. 40–44). Morphology of the coralloid buds was essentially identical to that of the vesicles completely enclosed within the matrix (compare Fig. 41 with Fig. 8). In haustorial stages the vesicular buds in the extrahaustorial membrane were present around the haustorial tips, whereas at the haustorial flanks towards the haustorial base they were increasingly substituted by coralloid matrix vesicles that were completely separated from the extrahaustorial membrane (serial sections not illustrated).

Many Golgi bodies and vesicles were located around the developing matrix (Figs. 16, 35–39). Golgi bodies were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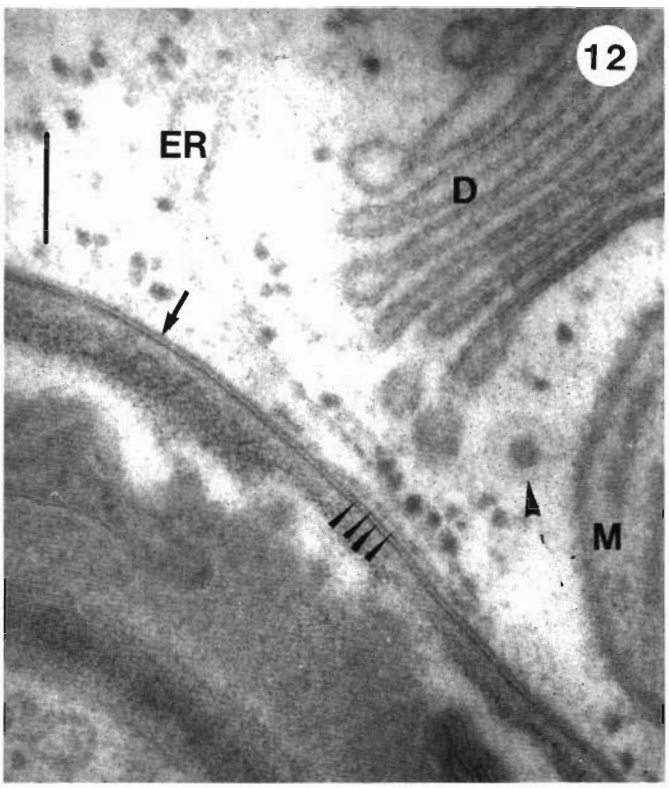
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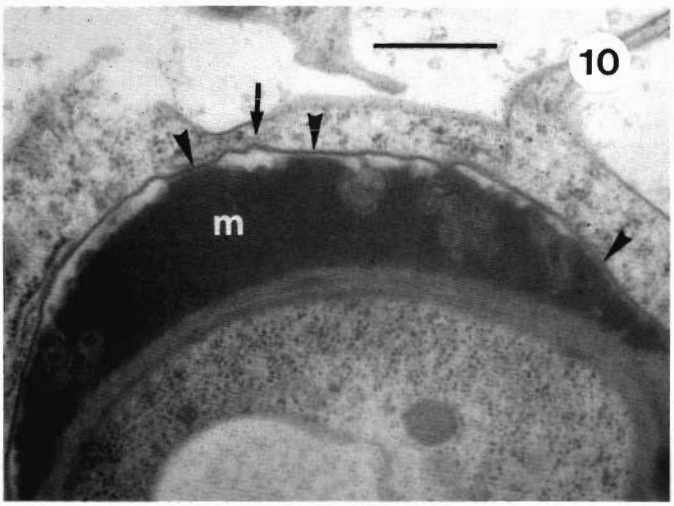
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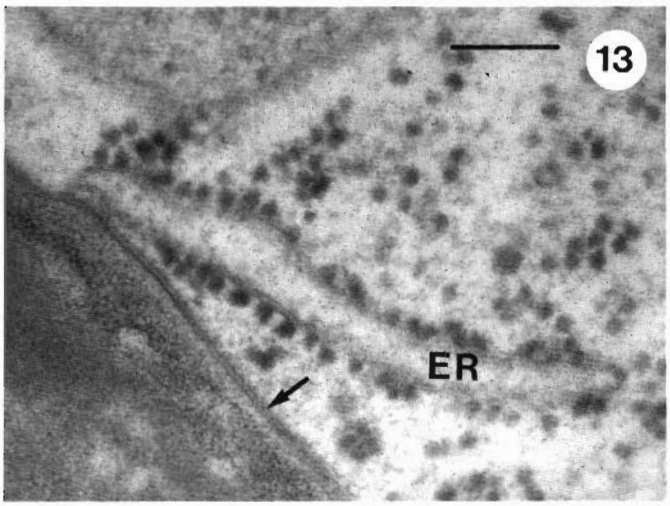
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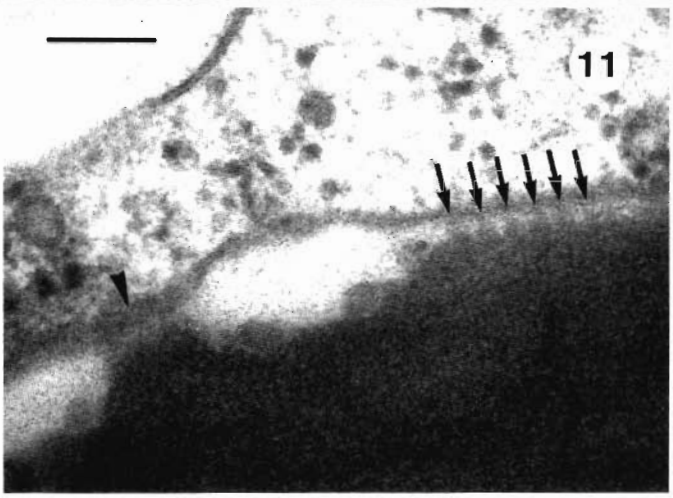
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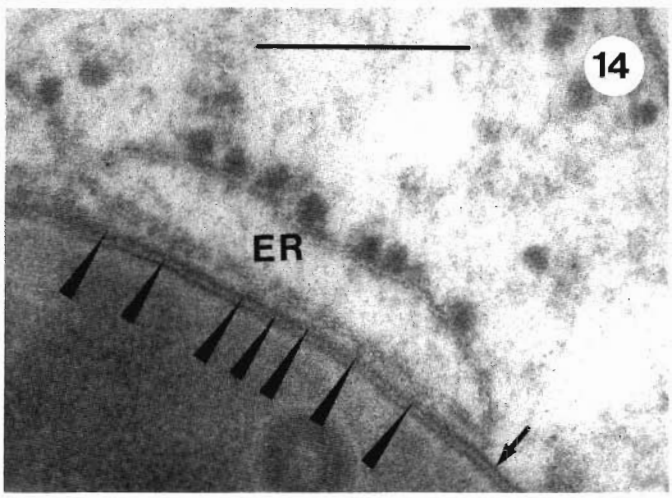
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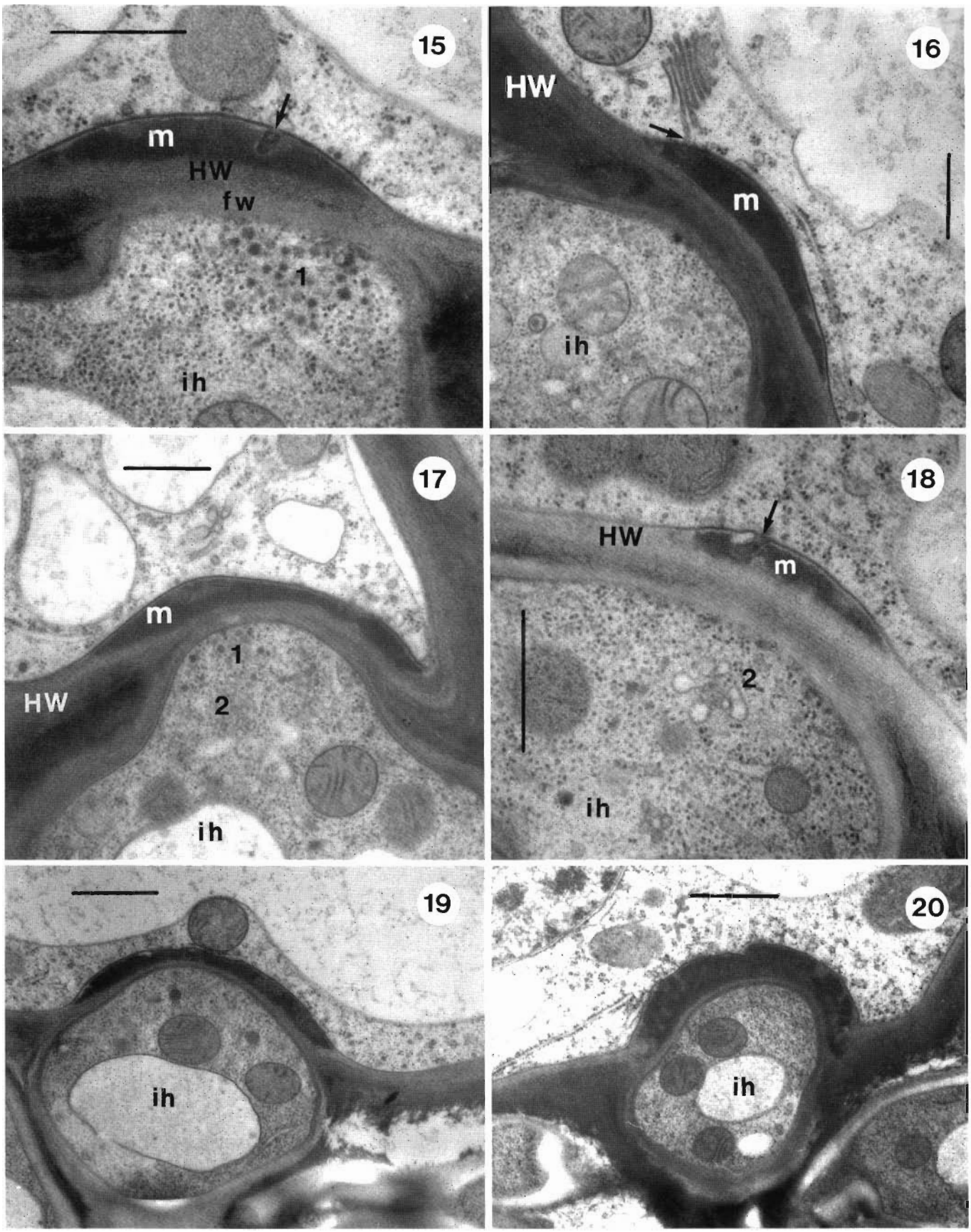
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