Ultrastructural Changes in Pepper Cells in an Incompatible Interaction with Phytophthora infestans

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ABSTRACT

Hypersensitive death of the first infected host cells was detected 4 hours after suspensions of Phytophthora infestans zoospores were injected into cavities of ripening fruit of sweet pepper (Capsicum frutescens). After 24 hours, many invaded host cells bordering the fruit cavity and some in the second layer, appeared to have reacted hypersensitively, while other invaded and adjacent uninvaded cells in the first and second layers were responding more slowly. In these cells, cell border lesions were observed between the host cell wall and invaginated plasmalemma prior to invasion and were associated with penetrating haustorial pegs during invasion. The reactivated cytoplasm of these cells was predominantly composed of smooth endoplasmic reticulum, polyribosomes, rough ER, ribosomes, and mitochondria. Haustorial contact with this cytoplasm was brief and a separation zone, containing vesicles and cytoplasmic fragments, was formed between the extrahaustorial matrix and the retreating extrahaustorial membrane. This zone resembled the cell border lesion matrix and was considered to be a similar cellular response. Disruption of the tonoplast membrane and fragmentation of the remaining cytoplasm eventually led to cell death. The first two-to-three cell layers bordering the fruit cavity were invaded during the 36 hours following zoospore inoculation. Most hyphae began degenerating between 36 and 48 hours and were dead after 7 days. Cells around the infected tissue slowly degenerated and possessed swollen nuclei, numerous lipid bodies, and chloroplasts with lipid-like granal deposits.

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In an earlier report, ultrastructural changes during invasion of pepper fruit pericarp cells by Phytophthora capsici were described (11). Most striking of these were the rapid penetration (2-4 hours) of host cells with distinct signs of their physiological reactivation, the generation of large numbers of ribosomes and endoplasmic reticulum followed almost immediately by cell death and tissue disorganization, and the apparently unimpeded growth of the fungus. Other studies have shown that during incompatible interactions with various non-pathogens of pepper, inhibitory concentrations of the phytoalexin, capsidiol, are induced, only low non-inhibitory concentrations accumulate in the compatible interaction just described (21). Evidently these events either are not associated with capsidiol formation or any other defensive mechanism, or the rapidity of cytoplasmic disorganization renders them ineffective. An examination has now been made of several incompatible interactions in an attempt to demonstrate ultrastructural events that may be associated with resistance and possibly with capsidiol induction. This paper describes results obtained with an isolate of Phytophthora infestans (Mont.) de Bary originally isolated from tomatoes, but non-pathogenic on peppers.

MATERIALS AND METHODS.—P. infestans, isolated locally from tomatoes, was grown on V8 juice agar in Petri dishes at 18 C. After 10-12 days of incubation, plates were flooded with 6-8 ml of sterile distilled water at 15 C and incubated for 75 minutes at 12 C. The resulting zoospore suspensions were filtered through Whatman’s No. 54 filter paper and zoospore concentrations were noted.

Fruits of greenhouse grown Capsicum frutescens L. (C. annuum L. var. grossum Keystone Resistant Giant), were harvested when half ripe and inoculated, as described previously (21), by injection of zoospore suspensions (1-4 x 10^6 spores) into their cavities. They were incubated for the periods indicated below at 18 C, (approximately the optimum temperature for in vitro growth of the fungus) and then opened and the diffusates removed. Pieces of tissue in the contact area were cut from the fruit and fixed for 2 hours in 2% formaldehyde and 2.8% glutaraldehyde mixed (1:1, v/v) in 0.05M phosphate buffer at pH 7.6. After being washed overnight in phosphate buffer, the tissues were postfix in a 1% solution of osmium tetroxide in phosphate buffer for 2 hours, rinsed several times in Kellenberger’s buffer, and then stained for 0.5-1.0 hour in Kellenberger’s uranium solution (13). After dehyrdration in an ethanol series, all samples were embedded in Spurr’s resin (18) and incubated at 70 C for 1-3 days. Penetration sites and invaded areas were located by light microscopy before ultrathin sectioning. Sections were stained in a 0.25% solution of lead citrate for 45 seconds (23) and examined with a RCA (EMU3F) electron microscope. The terminology adopted by Bushnell (2) has been used in detailed descriptions of fungal haustoria.

RESULTS.—Light microscopy.—Encysted zoospores, germ-tubes, and appressoria were common on the surface bordering the pepper fruit cavity 4 hours after inoculation. After 1 day, a number of light- to dark-brown pepper cells were seen where the network of germ-tubes was particularly dense. This occurred mainly in depressions, which constituted approximately half of the corrugated, diamond-patterned inner surface area. All ultrastructural studies in this paper were limited to these regions, where most infection seemed to occur. The proportion of brown cells continued to increase in these regions and after a week, the inner surface of the fruit cavity appeared dark brown to the naked eye. Ripening pepper fruit had usually turned red by this time, except in areas where the inoculum was in contact with the tissue, which remained green.

Noninfected control host cells.—Most cells had a large central vacuole with a thin layer of cytoplasm containing a few chloroplasts, mitochondria, scattered ribosomes, and fragmentary rough endoplasmic reticulum (ER) lining the cell wall. Nuclei were usually smooth in outline and in some cells the cytoplasm appeared aggregated and contained a few lipid droplets (11).

Observations 4 hours after inoculation.—Fungal hyphae had invaded the walls and vacuoles of cells bordering the fruit cavity (Fig. 1). Sections through most hyphae revealed cytoplasm dense with ribosomes and containing ER, lomasomes and numerous mitochondria. Invaded host cells had reacted hypersensitively, and contained disorganized, vesiculated cytoplasm and lipid droplets (Fig. 1). Papillalike structures, surrounded by the host plasmalemma, intruded into the host cell vacuole at points where intercellular hyphae appeared to penetrate the cell (Fig. 1, 2) and large fungal lomasomes were usually associated with the fungal wall at the interface (Fig. 2).

Observations 1.0 day after inoculation.—Intercellular hyphae and globose, lobed, nucleate haustoria with elongated hyphal-like projections (Fig. 3) were observed in the first two cell layers bordering the fruit cavity. The fungus usually appeared normal (Fig. 3, 5, 8, 13, 18), but in some hypersensitive cells, the cytoplasm was degenerating (Fig. 4).

Many of the first invaded cells had reacted hypersensitively and contained necrotic cytoplasm (Fig. 4, 9), while other subsequently invaded cells and adjacent uninjured cells were in various stages of a different type of response. The activated cytoplasm in most of these cells consisted predominantly of dilated, vesiculated smooth ER and polyribosomes with numerous normal and degenerating mitochondria and some rough ER (Fig. 7, 10, 15, 18). Microbodies containing crystalline inclusions, similar to those observed in control cells and in tissues infected with P. capsici (11), were seen occasionally near groups of mitochondria (Fig. 10). Originally thought to have been formed from bulges in chloroplast membranes (11), it now seems likely that these microbodies developed from the similarly sized mitochondria, with which they were closely associated. On two occasions, degenerating mitochondria were partly surrounded by one or two layers of undilated, smooth ER, which may have been extensions of their outer membranes (Fig. 11), indicating their intimate involvement in cellular metabolism.

In uninjured, reactivated cells, the host cytoplasm and plasmalemma were commonly separated from the cell wall (Fig. 10). Vesicles, resembling smooth ER swellings in the host cytoplasm, were seen in the separation zone and intimately associated with the plasmalemma (Fig. 10, 12). At certain points, this separation was very obvious and accumulations of membrane-bound vesicles and dark, irregular inclusions were usually visible between the
host plasmalemma and cell wall (Fig. 9, 15, 16). Some of the inclusions were closely associated with the cell wall (Fig. 13) and appeared to have arisen from the breakdown of its outer layers. Large cytoplasmic fragments were also occasionally observed in the separation zone (Fig. 16). These blister-like cell border lesions were often seen opposite pits in the cell wall, the plasmodesmata being occupied by elongated deposits with the same staining density as adjacent lesion inclusions (Fig. 13, 16). Small accumulations of lesion material were seen opposite the pit areas in the neighboring hypersensitive cells, indicating either a limited lesion response in the cell before death, or the movement of material through the plasmalemma. Golgi bodies were evident in the host cytoplasm near some lesions (Fig. 14).

Haustorial pegs penetrating these activated cells were surrounded by enlarged cell border lesions (Fig. 17, 18). A highly invaginated host plasmalemma separated the lesion matrix, containing vesicles and dark irregular bodies, from the host cytoplasm. Mitochondria, chloroplasts and nuclei with irregular outlines were commonly observed near these encapsulating cell border lesions (Fig. 17, 18). The cytoplasm of these cells appeared to be fragmenting into the cell vacuole, apparently due to a breakdown of the tonoplast membrane. One intruding hypha was also observed sheathed with a dark layer, which was associated with the host cell wall (Fig. 18). The similarity in staining density between this layer and the previously described papillae observed 4 hours after inoculation (Fig. 2), suggested that it was a sheath-like papilla extension.

In activated cells invaded by the fungus, cell border lesions were discernible around haustorial necks (Fig. 3). The host cytoplasm was usually separated from the haustorium (Fig. 3), but contact was close in some areas, especially around the hyphal-like protubercances (Fig. 3, 8), and where thin layers of fragmenting cytoplasm were attached to the main haustorial body (Fig. 5). Cytoplasmic fragments and vesicles, which were usually embedded in a narrow, irregular, meandering electron-lucid matrix layer, lay in the separation zone between the haustorial lobes and the bulk of the host cytoplasm (Fig. 5). A distinct layer, with the same staining density as the previously described papilla (Fig. 2) and its possible sheath-like extension observed around penetrating haustorial pegs (Fig. 18), covered the haustorial wall (Fig. 6, 8) and because of its position has been termed the extrahaustorial matrix. The invaginated host plasmalemma or extrahaustorial membrane appeared to keep its continuity (Fig. 6, 7, 8), but in many invaded cells the tonoplast membrane was discontinuous and the main body of the host cytoplasm was fragmenting into the cell vacuole. Long profiles of smooth ER, often in parallel arrays with vesicular swellings, scattered ribosomes, Golgi bodies, and mitochondria were common features of the bulk of host cytoplasm (Fig. 7).

A few cells adjacent to invaded cells contained large areas of cytoplasm densely packed with ribosomes. Parallel layers of rough ER with regularly arranged ribosomes were common features of these cells (Fig. 16).

Observations 1.5 days after inoculation.—Hyphae were observed in the first two, and occasionally the third, cell layers bordering the fruit cavity. The fungal cytoplasm generally stained lighter and was more vacuolated than at 1.0 day after inoculation. The organelles appeared normal in many profiles, but in others the membranes bordering large vacuoles were discontinuous and the cytoplasm was disorganized and fragmenting (Fig. 19). Similarly degenerating fungal cytoplasm had been observed occasionally 1.0 day after inoculation (Fig. 4).

Host cells invaded by haustoria were generally necrotic with rounded, deep staining nuclei. Sometimes, large areas of fine granular deposits separated the remains of the plasmalemma from the cell wall (Fig. 19). Large irreparable cell border lesions, apparently penetrated by haustoria, were seen in other cells. In one section, a cell border lesion with an irregular outline was observed near a slightly vacuolated and disintegrating intercellular hypha. The relationship between the host cytoplasm and lesion suggested that fragments of cytoplasm had been incorporated into the lesion matrix. A host nucleus was closely associated with this possible penetration site (Fig. 20).

Observations 2.0 days after inoculation.—Hyphae were present in the first two-to-three cell layers bordering the fruit cavity. All invaded host cells contained the diffuse, disorganized remains of necrotic host cytoplasm. Although some fungal cytoplasm appeared normal, a greater proportion than at 1.5 days showed signs of vacuolation and degeneration. Lipid bodies were evident in most hyphae and dark staining, lens-shaped deposits were frequently seen sandwiched between ER membranes. These deposits, which often bordered vacuoles in degenerating hyphae, were also observed 2.5 days after inoculation (Fig. 21).

Observations 2.5 days after inoculation.—As before, hyphae were confined to the first two-to-three cell layers. The dark staining, lens-shaped deposits were common features between ER membranes in the vacuolated, disintegrating fungal cytoplasm (Fig. 21). The highly invaginated fungal plasmalemma had separated from the fungal wall in places. One haustorium in a dead host cell appeared completely encapsulated by a very large cell.

Fig. 1-4. Phytophthora infestans in pepper cells bordering the fruit cavity. (Fig. 1, 2) Four hours after zoospore inoculation. 1) Infection hypha in cell wall between two hypersensitively degenerating host cells. Note nucleus migrating from appressorium, papillalike structure at point of contact between infection hypha and vesiculated host cell cytoplasm (arrow), and haustorium. (×5,200). 2) Magnification of papillalike structure showing surrounding host plasmalemma (arrows) and associated fungal lomosome. (×3,180). (Fig. 3, 4) One day after zoospore inoculation. 3) Host cytoplasm separating from main body of globose, nucleate haustorium with hyphal-like projection. Note lesionlike reaction of host cytoplasm (arrows). (×6,200). 4) Degenerating intercellular hypha and haustorial neck. Note deposit on haustorial wall (arrow) and absence of lesion material around cellular penetration site. (×13,000). Legend: A = appressorium; CV = cell vacuole; CW = cell wall; FC = pepper fruit cavity; FL = fungal lomosome; FN = fungal nucleus; H = haustorium; HC = hypersensitive cell; IH = intercellular hypha; and P = papillalike structure.
Fig. 5-8. Invaded pepper cells one day after inoculation with zoospores of *Phytophthora infestans*. 5) Host cytoplasmic vesicles and fragments, including a mitochondrion, embedded in an electron opaque layer (arrows) in separation zone between haustorium and bulk of host cytoplasm. Note the thin layer of fragmenting host cytoplasm bordering haustorium. (×8,200). 6) Magnification of fragmenting host cytoplasm bordering haustorium. Note distinct extrahaustorial matrix layer (black arrows) between extrahaustorial membrane (white arrows) and haustorial wall. (×63,700). 7) Scattered ribosomes, polyribosomes and smooth endoplasmic reticulum with vesicular swellings in bulk of activated host cytoplasm surrounding separation zone. Note extrahaustorial membrane bordering separation zone. (×49,400). 8) Profile of hyphalike haustorial projection in host cytoplasm. Note the separation of the extrahaustorial membrane (white arrows) from the extrahaustorial matrix (black arrows) along most of its length and Golgi body and fungal lomasome in haustorium. (×32,400). Legend: CV = cell vacuole; CW = cell wall; FL = fungal lomasome; FN = fungal nucleus; G = fungal Golgi body; H = haustorium; HW = haustorial wall; IH = intercellular hypha; M = host mitochondrion; and SZ = separation zone.
Fig. 9-15. Pepper cells 1 day after inoculation with zoospores of Phytophthora infestans. 9) Necrotic remains of host cytoplasm in invaded hypersensitive cell. Note dark staining haustorium (middle left) and blister-like cell border lesion in neighboring second layer cell (arrow). (× 3,130). 10) Reactivated host cytoplasm containing degenerating mitochondria, microbody with crystalline inclusion, polyribosomes, rough endoplasmic reticulum (ER) and vesicles believed to be smooth ER swellings. Note one such vesicle (arrow) associated with plasmalemma separating from cell wall. (× 21,400). 11) Smooth ER layers partly surrounding a degenerating mitochondrion in reactivated host cytoplasm. Note possible point of contact with outer double mitochondrion membrane (arrow). (× 37,000). 12) Separation of host cytoplasm (below) from cell wall. Note close association of smooth ER with plasmalemma. (× 30,500). 13) Cell border lesion adjacent to a pit in the cell wall. A smaller lesion is discernible in the hypersensitive cell (left). Note dark staining haustorium in dead cell, dark elongated deposits occupying the plasmodesmata (arrow), vesicular lesion inclusions, and host cytoplasm fragmenting in cell vacuole (right). (× 15,300). 14) Golgi body in host cytoplasm near cell border lesion. (× 70,000). 15) Cell border lesion with vesicular inclusions. Note polyribosomes and swollen smooth ER in reactivated host cytoplasm. (× 22,000).

Legend: CI = crystalline inclusion; CV = cell vacuole; CW = cell wall; FC = pepper fruit cavity; H = haustorium; HC = hypersensitive cell; L = cell border lesion; and M = host mitochondrion.
border lesion, which was bordered by the thickened, densely staining remains of the extrahaustorial membrane (Fig. 22).

Observations 3.0 days after inoculation.—Almost all fungal cytoplasm appeared vacuolated, granular or vesiculated, and mitochondria were in an advanced stage of degeneration (Fig. 23, 24). Occasionally, however, a comparatively normal haustorium with apparently functional organelles was observed in a necrotic or degenerating cell (Fig. 25).

Host cells adjacent to the necrotic cells, which marked the limit of fungal invasion, possessed highly invaginated and swollen nuclei (Fig. 28). The vesiculated cell cytoplasm contained mitochondria in various stages of internal breakdown, numerous lipid bodies and chloroplasts with dark staining lipid-like deposits in their grana (Fig. 26). These deposits were occasionally so extensive that the chloroplasts were almost unrecognizable (Fig. 27).

Observations 7.0 days after inoculation.—Most fungal cytoplasm had almost (Fig. 30), or completely degenerated (Fig. 29). Occasionally, however, haustoria were seen with vacuolated, distitiginating cytoplasm more typical of haustoria 1.5 to 2.0 days after inoculation.

Fragmented remains of the host cytoplasm were present in some invaded cells, but others appeared empty. Some cell walls were darkly stained.

DISCUSSION.—Light and electron-microscope studies indicate clearly that P. infestans is able to infect fruit tissues of the nonhost plant, sweet pepper (Capsicum frutescens). The sequence of events, involving zoospore encystment, germ-tube elongation, appressorial formation, infection, haustorial development and death of invaded host cells can occur very rapidly, within as few as 4 hours following inoculation (Fig. 1). Invasion of the second and third cell layers bordering the fruit cavity, however, took up to 1.5 days following inoculation, and did not extend to deeper layers. Considering the speed of infection in the first few hours, the rate of hyphal growth must have slowed immediately following the hypersensitive death of the first penetrated cells. Although a few hyphae and haustoria still appeared normal after 3.0 days (Fig. 25), the great majority showed signs of deterioration between 1.5 and 2.0 days following inoculation (Fig. 19, 20), and some appeared degenerate even after 1.0 day (Fig. 4). Most hyphae and haustoria had almost completely degenerated after 7.0 days (Fig. 29, 30). While normal haustoria were often seen in necrotic host cells (Fig. 1, 9, 13), degenerating haustoria were never seen in healthy cells. This would strongly suggest that, contrary to the view expressed by Kirdly et al. (14) and in agreement with ultrastructural studies of *Bremia lactucae* on lettuce by Maclean et al. (15), host cell death precedes hyphal deterioration.

The hypersensitive reaction to invasion of the first infected cells, which is believed to have occurred during penetration must have been extremely rapid and involved the vesiculation and fragmentation of the host cytoplasm and the formation of lipid droplets (Fig. 1, 9). Blisterlike lesions bordering the walls of surrounding noninvaded physiologically reactivated cells (Fig. 9, 15) and associated with penetrating haustorial pegs in other similarly reactivated cells (Fig. 17, 18), resembled structures termed lomasomes (6), cell wall lesions (7), reaction material (16), papillae (22), sheaths (1, 10), callose deposits (9, 10), and extrahaustorial matrices (17), described in other interactions. Most of the inclusions observed in the lesion matrix seemed to originate from degenerating fragments of host cytoplasm, but the dilated smooth ER system in the reactivated cell seemed closely associated with the host plasmalemma during separation from the cell wall (Fig. 10, 12) and may have been contributing some material. Golgi bodies, which were evident around some developing lesions (Fig. 14), and smooth ER, have been linked to carbohydrate metabolism and cell wall formation (3), and it is possible that polysaccharides were being secreted. Heath and Heath (10) have reported that lesion-like deposits and haustorial sheaths, formed in an immune interaction between cowpea (*Vigna sinensis*) and cowpea rust (*Uromyces phaseoli var. vignae*), reacted as callose in histochemical tests. The enzymic action of other cellular secretions on certain cell wall components could account for the apparent incorporation of a few wall fragments into the lesion matrix as inclusions (Fig. 13).

The location of cell border lesions in uninjured cells in areas adjacent to intercellular hyphae or to haustoria in neighboring cells suggests that fungal metabolites may stimulate their formation. Victorin, the toxin produced by *Helminthosporium victoriae*, has been reported to induce wall lesions in susceptible oat roots (8). The lesion response at pits (Fig. 13, 16), may, therefore, have been an attempt by the cell to isolate itself from fungal toxins or toxic products of the host-parasite interaction.

Cell border lesions were not evident in most of the first infected, hypersensitive cells (Fig. 1, 4), though small deposits of lesion-like material, which may have arisen before cellular collapse, were observed near some pits (Fig. 13). In these cells, dark deposits, which appeared to have been the remains of papillae or their sheath-like

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**Fig. 16-20.** Pepper fruit cells. (Fig. 16-18) One day after inoculation with zoospores of *Phytophthora infestans*. 16) Cell border lesion adjacent to pit in cell wall. Note dark lesion inclusions occupying plasmodesmata (arrow) and ribosome-dense host cytoplasm with parallel layers of rough endoplasmic reticulum (ER). (× 37,500). 17) Host nucleus associated with cellular penetration site. Note large cell border lesion encapsulating intruding haustorial peg and haustorium (above) in adjacent dead cell. (× 4,200). 18) Magnification of cell border lesion encapsulating intruding haustorial peg and haustorium (above) in adjacent dead cell. (× 23,100). 19) Magnification of cell border lesion encapsulating intruding haustorial peg and haustorium (above) in adjacent dead cell. (× 23,100). 19) Host nucleus associated with cellular penetration site. (× 13,600). Legend: C = chloroplast; CV = cell vacuole; CW = cell wall; D = diffuse deposit; H = haustorium; IH = intercellular hyphal; L = cell border lesion; N = host nucleus; M = host mitochondrion; and SG = starch granule.
extensions, were seen on haustorial neck walls (Fig. 4) and near cellular penetration sites (Fig. 1, 2). Similar papillae were seen previously in pepper fruit cells during the compatible interaction with Phytophthora capsici (11), and it is possible that their formation is therefore a nonspecific response to penetration. The similarity in staining density and position on haustorial walls between papillae (Fig. 2), papilla deposits (Fig. 4) and extrahaustorial matrices (Fig. 6, 8) suggests that the extrahaustorial matrix is a sheath-like extension of the papilla. The absence of the extrahaustorial matrix in hypersensitive cells may be due to cellular collapse before full haustorial development. The disappearance of the papilla in cell border lesion-forming cells, could be caused by its incorporation into the developing lesion, possibly as inclusions, as is indicated by Fig. 18.

Most penetrating haustorial pegs (Fig. 18) were apparently able to break through the cell border lesion (but not the invading host plasmalemma/extrahaustorial membrane) and develop into mature haustoria, the remains of the lesion forming a collar around their necks (Fig. 3). Weak penetration attempts by slow growing haustorial pegs, originating from intercellular hyphae evidently just prior to their degeneration, may however have been stopped at cell border lesions before haustorial expansion (Fig. 20).

The nucellate, globose, haustoria with hyphal-like projections surrounded initially by extrahaustorial matrices (Fig. 3, 5, 6, 8) resembled haustoria of obligate fungal parasites (5) and were more elaborate than the small, anucleate, globose to peglike structures with few organelles seen by Ehrlich & Ehrlich (4) in P. infestans infected potato cells. Few haustoria were seen closely associated with viable host cytoplasm, indicating that the period of intimate contact between the thin extrahaustorial matrix layer and extrahaustorial membrane after penetration of the cell border lesion (Fig. 8) must have been brief. The separation of the extrahaustorial membrane from the main haustorial lobe (Fig. 3, 5) and later from the hyphal-like projections and the fragmentation of host cytoplasm in the separation zone (Fig. 5, 6) appeared to be an expansion of the cell border lesion response. A large amount of the lesion material that was produced in separation zones in many cells before the host cytoplasm disorganized, appeared to partly or completely encapsulate haustoria (Fig. 22, 25). A diagrammatic representation of the possible haustorial penetration, expansion and subsequent encapsulation process in these cells is illustrated in Fig. 31.

The amount, organization, and distribution of the ER in the cell border lesion-forming cells, as compared to control cells, suggested that increases occurred. Nuclei with irregular outlines, chloroplasts, and mitochondria were often observed near large lesions (Fig. 17, 20) indicating their possible movement to penetration sites. Large accumulations of protoplasm were not observed near cellular penetration sites in the compatible interaction with P. capsici (11). There, activated cells were shown to possess a ribosome-dense cytoplasm with parallel layers of rough ER. Although the response of some uninvaded, reactivated cells in the incompatible interaction was similar (Fig. 16), in the majority of cases it was not (see above). This may well indicate a fundamental difference in the metabolic response in the two types of interaction.

All penetrated cells eventually degenerated, though this appeared to have been less rapid in the cell border lesion-forming cells than in the initially infected hypersensitive cells. Physiologically reactivated uninvaded cells adjacent to degenerated cells delimiting the infected tissues underwent a much slower degeneration which was associated with the accumulation of lipid-like deposits in chloroplast grana and their transformation into fat bodies (Fig. 26, 27). Although it is possible that these chloroplasts were differentiating into chromoplasts, the process differed from previously described transformations in pepper fruit cells, where carotene fibrils and osmiophilic droplets built up in the chloroplast stroma (19, 20). Also, the transformation did not usually occur in tissue adjacent to the interaction site, as is evidenced by a delay in pepper fruit ripening in the P. infestans inoculum region. As this was visible on the fruit exterior, the normal metabolic processes in cells were therefore affected in tissue as far as 5 mm from the inner fruit cavity surface interaction zone.

It is evident from the events described here that P. infestans is well equipped to penetrate pepper fruit cells as rapidly as the pathogen, P. capsici (11). Incompatibility is expressed at the cellular penetration stage during which disintegration of the host cytoplasm occurs in what appears to be a hypersensitive response. This reaction differed from the compatible response to P. capsici, where the production of large numbers of ribosomes and rough ER was stimulated before the rapid disorganization of the host cytoplasm. A major difference between the two interactions followed as invasion extended to deeper layers. While P. capsici rapidly spread to other cells, apparently unimpeded and stimulating similar responses,
P. infestans appeared to lose vigor, reactivated cells in a different manner, and subsequent intercellular growth took place relatively slowly until finally it stopped. The significance that production of the phytoalexin capsidiol may have in this response is discussed in detail in the following paper (12).

Fig. 31-(a to e). Diagrammatic interpretation of the sequence of ultrastructural events associated with P. infestans haustorial penetration and expansion in reactivated cell border lesion-forming pepper cells. a) Cell border lesions arise near an intercellular hypha and at a pit. Lesion material occupies the plasmodesmata (see Fig. 13, 16). A papilla forms around hyphal tip at penetration point. b) Penetrating haustorial peg is surrounded by a sheath-like extension of the papilla formed at the invaginating plasmalemma (extrahaustorial membrane)—fungal cell wall interface. A developing cell border lesion is incorporating sheath fragments into the lesion matrix as it slowly expands around the relatively fast growing haustorium. The host nucleus and associated cytoplasm congregates near the penetrating haustorium (see Fig. 17, 180). c) The cell border lesion at the penetration site forms a collar around the neck of the expanded haustorium. The extrahaustorial membrane is separating from the main haustorial lobe but contact is still maintained around the growing haustorial projection. Contact between the extrahaustorial membrane and fungal wall is brief and only a thin extrahaustorial matrix layer is formed at the interface (see Fig. 3, 8). The tonoplast membrane begins to break down. d) Vesicles and fragments of host cytoplasm are shed into the separation zone between the haustorium and the retreating extrahaustorial membrane. Most of these originate from a thin layer of cytoplasm lining the haustorial wall. In the later stages of this disintegration, the separation zone resembles the cell border lesion matrix and therefore appears to be a similar cellular response (see Fig. 5, 6, 25). Further disruption of extrahaustorial membrane leads to the fragmentation of cytoplasm into the cell vacuole. e) Encasement of haustorium by lesion material formed in separation zone by retreating, disorganizing host cytoplasm. Remains of extrahaustorial membrane and cytoplasm lie around encapsulation (see Fig. 22). Legend: C = reactivated cell cytoplasm; CV = cell vacuole; CW = cell wall; DC = dead cell; EM = extrahaustorial membrane; EN = lesion-like encasement; H = haustorium; HP = haustorial projection; L = cell border lesion; P = papilla; PM = plasmalemma; PT = plasmodesmata; S = sheath-like extension of papilla/extrahaustorial matrix; SZ = separation zone; and T = tonoplast membrane.
LITERATURE CITED


