Fine Structure of Puccinia carthami and the Ultrastructural Nature of Exclusionary Seedling-Rust Resistance of Safflower

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ABSTRACT

The fine structure of intercellular hyphae and intracellular haustoria of *Puccinia carthami* closely parallels that of other *Puccinia* spp. Lomosomes occurred both in haustoria and hyphal cells, and were believed to be involved in cell wall deposition rather than absorption. No channels through the haustorial wall connecting the haustorial cytoplasm and the sheath matrix or host cytoplasm were observed. Contrary to published reports for other obligate parasites, the sheaths surrounding mature haustoria of *P. carthami* were not of a single amorphic structure, but were composed of three distinct, perhaps functional, regions.

The exclusionary seedling-rust resistance of the safflower, *Carthamus tinctorius,* Nebraska 1-1-5, resulted from cytoplasmic inhospitality of the cells of the perivascular region. Resistance was manifested by cytoplasmic collapse, degradation of the haustorial sheath, and the apparent deprivation of the haustorium of nutrients essential for glycogen synthesis. Glycogen was abundant in haustoria and hyphae in the perivascular region of Nebraska 8, a susceptible variety, but absent from these structures in the same region of Nebraska 1-1-5.

Crystal-containing microbodies were abundant in cells in the region where exclusionary-seedling-rust resistance was operative. The rapid disappearance of these bodies in rust-infected cells suggests that they may play an important role in incompatibility. Phytopathology 60:1157-1163.

This investigation was undertaken to compare haustorial development and morphological alterations induced in rust-infected cells of a compatible (susceptible) variety with an incompatible (resistant) variety, and to explore the ultrastructural nature of the exclusionary resistance of Nebraska 1-1-5 safflower.

Safflower, *Carthamus tinctorius* L., is the host of the rust fungus, *Puccinia carthami* Cda. Because of its macrocyclic-autoecious nature, *P. carthami* has two pathological phases, a seedling phase and a foliar phase (23, 24, 29). Most safflower introductions, selections, and varieties resistant to seedling rust are resistant to foliage rust (30, 32). Resistance to both phases is normally conditioned by the same genetic factors, and probably are physiologically related (32, 33). Nebraska 1-1-5, unlike other varieties and lines, is resistant to seedling rust but susceptible to foliage rust (32). This differential reaction to the two pathological phases suggested that seedling resistance of Nebraska 1-1-5 might be of an unusual nature. Light microscopical studies revealed that the seedling resistance of Nebraska 1-1-5 resulted either from the structure or from physiological properties of cells in the perivascular region of the hypocotyl which excluded rust hyphae from the vascular region (31).

Electron microscopy has contributed greatly to our knowledge of fungal structure and host-parasite interactions. Most fine structural research of host-parasite interactions has dealt with haustorial structure, host-parasite interface, and the morphological manifestations induced by haustoria in cells of a compatible (susceptible) system (3, 4, 8, 10, 14, 15, 19, 20, 22). Only Ehrlich & Ehrlich (7) and Shaw & Manocha (25) have studied the haustorial structure and morphological changes which occur in both incompatible cells (resistant) and compatible cells (susceptible) when attacked by a rust fungus.

MATERIALS AND METHODS.—Surface-sterilized seeds of *C. tinctorius* 'Nebraska 1-1-5', resistant to seedling rust incited by *P. carthami,* and 'Nebraska 8', susceptible to seedling rust, were planted in flats of steamed soil and maintained in a greenhouse at 23 ± 3 C. When the seedlings emerged, 50% were inoculated by pouring an aqueous suspension of rust teliospores directly over them; they were then placed in a moist chamber for 18 hr. The remaining 50% were left noninoculated.

Hypocotyls were harvested 4, 8, 12, and 18 days later. Slices 0.5 mm to 1 mm thick were fixed in either phosphate buffered 3% gluteraldehyde or 3% acrolein —3% gluteraldehyde, pH 7.0, then postfixed in 2% OsO_4 (2). The material was then stained in bulk with a saturated aqueous solution of uranyl acetate, dehydrated in a graded acetone series, and embedded in a 1:2 ratio of Epon A and Epon B (17). Sections were cut on a Porter Blum II ultra-microtome, counterstained with Reynold's lead citrate, and examined on a Zeiss EM-9A electron microscope.

RESULTS AND DISCUSSION.—*Ultrastructure of hyphae and haustoria.*—The ultrastructure of the intercellular hyphae and intracellular haustoria of *P. carthami* follow closely that described by Ehrlich & Ehrlich (9) and Shaw & Manocha (25) for other *Puccinia* spp. The haustoria and hyphae have single-zone cell walls ca. 800-1500 Å in thickness separated from the fungus protoplasm by well-defined undulating plasmalemma (sheath membrane) (Fig. 1, 2). Lomosomes (20) were

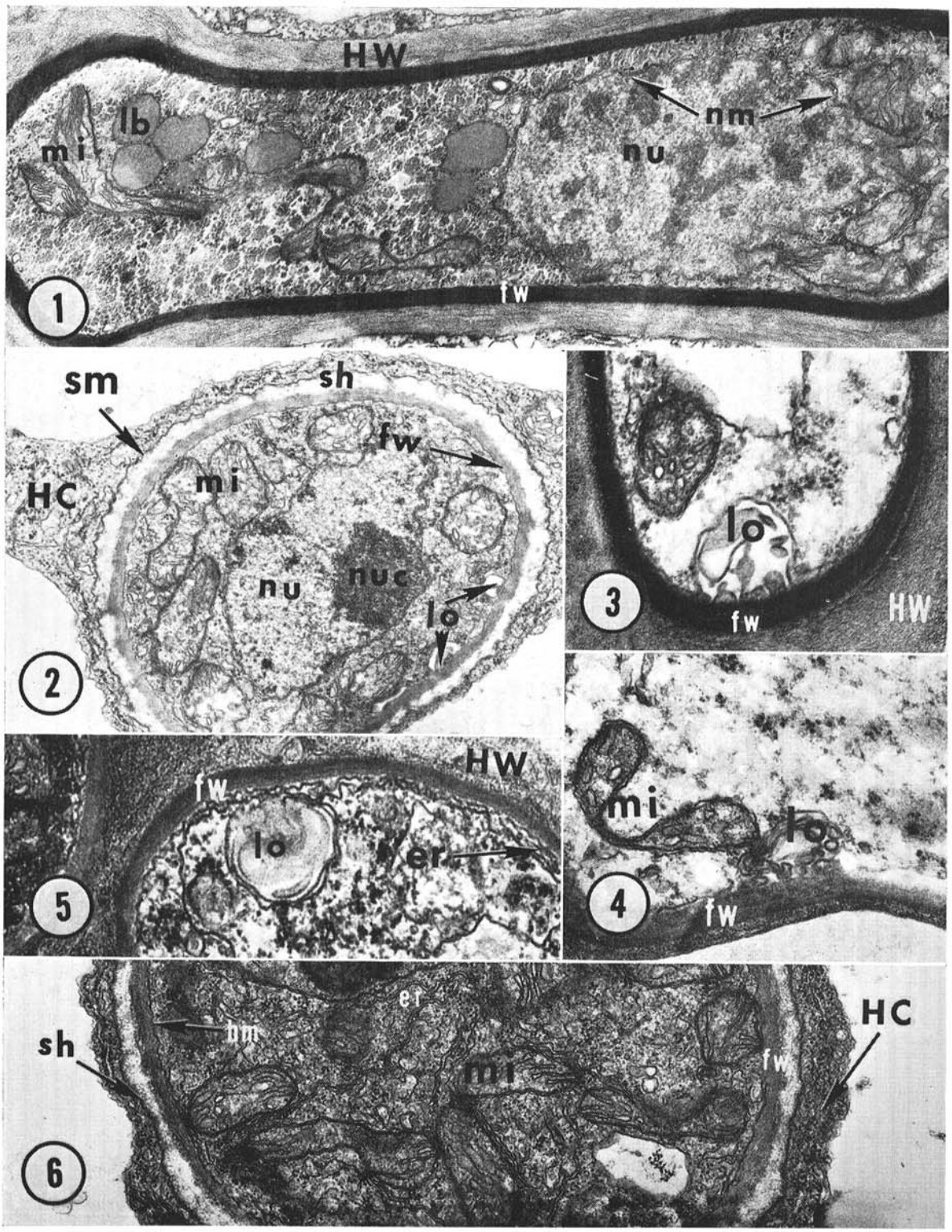

Fig. 1-6. **1)** Electron micrograph of an oblique section of an intercellular hypha, showing the general organization of the hyphal components (×21,000). **2)** Electron micrograph of a median section of an intracellular haustorium, showing the general arrangement of the haustorial components (×21,000). **3, 4, 5)** Section through large hyphal lomosomes showing their structural detail and association with the hyphal wall (×25,500). **6)** Electron micrograph of a median section of a young haustorium, showing the undulating haustorial membrane, haustorial wall, sheath, sheath membrane, an abundant endoplasmic reticulum and numerous ribosomes (×25,500). er = Fungus endoplasmic reticulum; fw = fungal wall; HC = host cytoplasm; HW = host cell wall; hm = haustorial membrane; lb = lipid body; lo = lomosomes; mi = mitochondria; nm = nuclear membrane; nu = nucleus; nuc = nucleolus; sh = sheath; sm = sheath membrane.

frequently observed between the undulating plasmalemma and the hyphal wall (Fig. 2); although normally small, the lomosomes were particularly large in some sections (Fig. 3, 4, 5). Lomosomes were observed in both hyphae and haustoria. Their high frequency in hyphae, organs which are not considered highly specialized absorptive, suggests that lomosomes do not function as suggested by Peyton & Bowen (22) as major absorptive bodies. On the other hand, the absence of dictysomes in both hyphae and haustoria implies that the lomosomes may play a role in cell wall deposition (28).

Similar cytoplasmic organelles were present in both the haustoria and the hyphae; thus, distinguishing these organs solely on the absence or presence of specific cytoplasmic organelles appears unlikely (Fig. 1, 2, 7). Mitochondria, the spherical and filamentous forms, were present in both haustoria and hyphae but were more abundant in the haustorial heads. The mitochondria were surrounded by a double limiting membrane. The inner membrane of the pair was invaginated to form cisternae which were either arranged longitudinally, horizontally, or transversely to the long axis. The endoplasmic reticulum and ribosomes were scarce in hyphal cells and mature haustoria, but were abundant in young haustoria (Fig. 6). Lipid bodies (Fig. 1, 7), although present in both hyphae and haustoria, were never as abundant as reported for *Phytophthora* spp. (7). The presence of lipid bodies increased with age, but could not be used solely as an indicator of age. Glycogen particles (Fig. 7, 8, 10) were extremely abundant in both mature haustoria and hyphae within the susceptible host. Their abundance may be used as a criterion for ascertaining haustorial age. Young haustoria are devoid of glycogen particles. As haustoria became increasingly active and mature they accumulate glycogen. Glycogen assimilation continues until sporulation begins, at which time it gradually disappears (Fig. 11). Glycogen particles are abundant not only in haustoria within cells of the susceptible variety but also in the intercellular hyphae (Fig. 8). Glycogen particles may move into the hyphae after being synthesized in the haustoria, or they may actually be synthesized in the hyphae after transport of essential materials.

As reported for *P. graminis* (9, 25), the haustoria of *P. carthami* contain a single nucleus which in some sections exhibits a distinct nucleolus (Fig. 2). The nuclear envelope appears to contain pores, as reported by Shaw & Manocha (25).

Aside from the qualitative relationship of cytoplasmic organelles in haustoria and hyphae, these structures bear little resemblance in gross morphology and function. The hyphae are intercellular and are tightly appressed to the cell walls of the host. The haustoria are capitate and intracellular. The haustoria are surrounded and separated from the host protoplasm by a plasma membrane (sheath membrane) which appears to be no more than the invaginated host plasmalemma. An irregular layer of heterogeneous material located on the haustorial side of the plasma membrane, exterior to the haustorial wall, is produced by the activity of the host, parasite, or interaction of both (Fig. 2, 6, 7, 10). This structure has been referred to by earlier workers as a sheath (4, 9, 26), a sack (16), an encapsulation (9, 10) or a zone of apposition (22). This definite delimiting structure, bounded by the haustorial wall and the host's plasma membrane, is a general feature of most, if not all, obligately parasitic fungi. Although different terminology has been used to describe this structure, we are in agreement with Bracker (4) who, in an effort to preserve order in terminology, proposed that the term "sheath" originally used by Smith (26) be applied to this structure.

The structure surrounding unbranched haustoria of *P. carthami* is similar to the structure surrounding the haustoria of other obligate parasites, and probably has a similar origin and function. Thus, the adoption of the term "sheath" appears appropriate. The opinion is divided concerning the origin of the sheath around haustoria of obligate parasites. Smith suggested that it was the accumulation of cellular debris from the cell wall of the host. Caporali (5) considered it to be pectic substances of haustorial origin. Ehrlich & Ehrlich (9) thought that it was composed of either metabolic products of either host or fungal origin or extended fungal cytoplasm. Shaw & Manocha (26), Berlin & Bowen (3), Peyton & Bowen (22), and Hirata & Kojima (16) support the view that the sheath is of host origin. McKeen et al. (19) believe that the sheath results from host-parasite interaction. Sheaths do not occur around haustoria found within cytoplasmic void laticifers (Fig. 9), although haustoria found there appear to be physiologically active. This suggests that if the sheath is not a product of the host cytoplasm, it at least is a product of host-parasite interactions as proposed by McKeen et al. (19). The hypothesis that the sheath originates from the interaction of host and parasite, and not by the action of either one independently, is also supported by structural variation and differentiation with age. Young haustoria are encased in a sheath of homogeneous structure (Fig. 6, 13). As the haustorium ages, increases in size, and becomes increasingly active, the sheath appears stratified with at least three prominent regions (Fig. 10). Fraymouth (12) found that sheaths of the Peronosporales varied in size, morphology, and texture (solid, liquid, and gel). The sheaths surrounding young haustoria of *P. carthami* are homogeneous with little evidence of substructure, and may be composed primarily of liquid. Those around mature haustoria (Fig. 10) appear to be composed of at least three definite regions, an outer one believed to be primarily liquid, an inner one perhaps of deposited by-products of physiological processes, and between the two an area permeated by electron opaque particles that may reflect physiological activities. Ehrlich & Ehrlich (7, 8) observed particulate material in the encapsulation around haustoria of *P. graminis,* and considered them indistinguishable from those found in the haustorial cytoplasm. The particles I observed in sheaths of *P. carthami* bear little resemblance to haustorial particles, and it is presumed that they have their origin elsewhere. We failed to observe channellike areas connecting the encapsulation with the haustorial cytoplasm as reported for *P. graminis* (8), but some of the debris observed in the outer regions of the sheath may be plasmadesmata (Fig. 10).

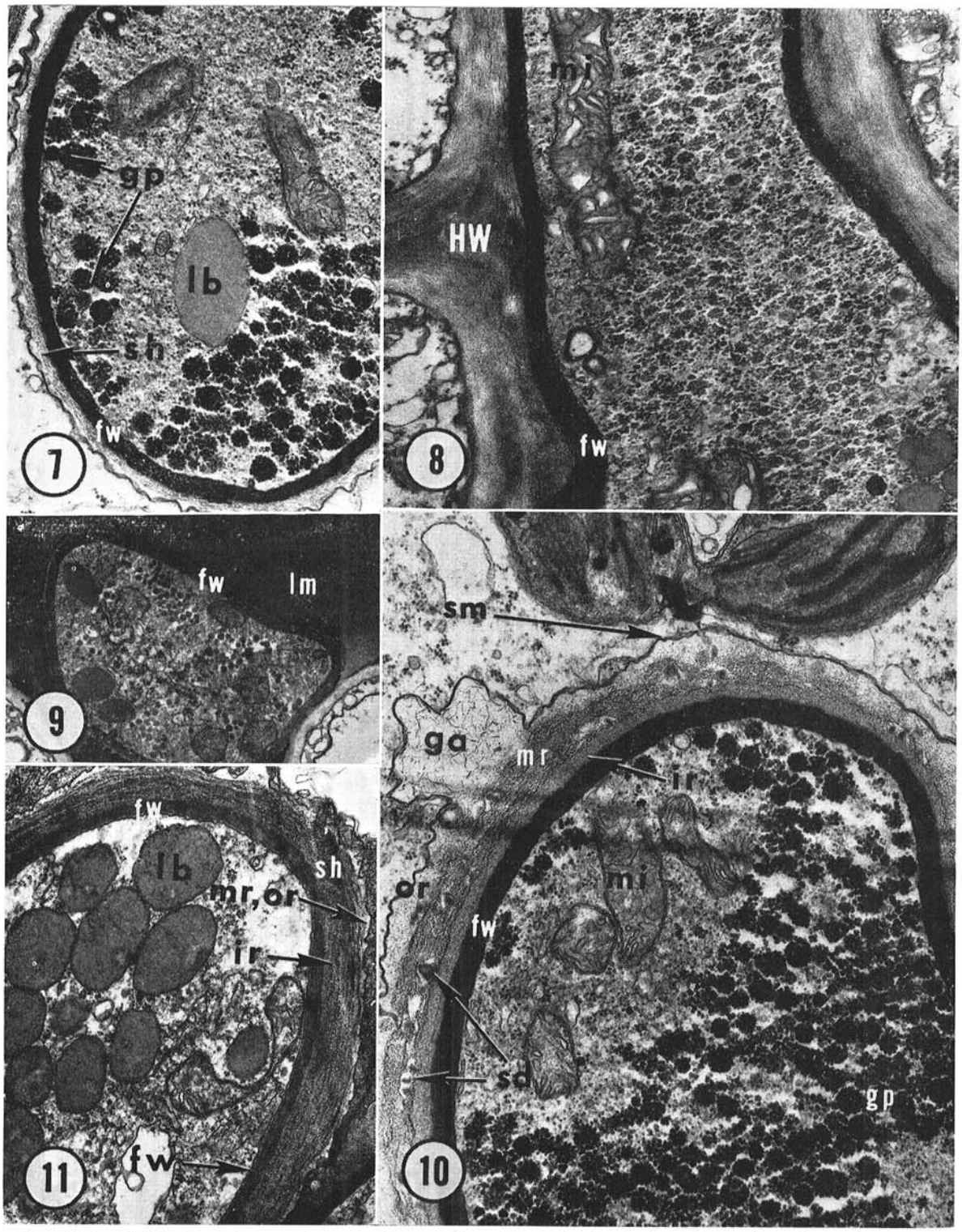

Fig. 7-10. **7)** Electron micrograph of a median section of an active haustorium. Soon after a haustorium becomes parasitically active in a compatible host cell, it begins to store glycogen (×21,000). **8)** Electron micrograph of a longitudinal section of an intercellular hypha, showing abundant glycogen particles that have either been synthesized there or moved from the haustorium (×25,500). **9)** Electron micrograph of a cross section of a haustorium which has penetrated a laticifer. Haustoria formed inside cytoplasmic-void laticifers lack sheaths (×9,250). **10)** Section of a mature haustorium inside a compatible cell. The sheaths surrounding such haustoria possess three distinct regions, an inner region, a middle region, and an outer region. What appear to be bits of debris, but may be sections of plasmadesmata, are frequently found in the matrix of the middle and outer region. Glandlike appendages frequently grow out from the outer region (×25,500). **11)** A section through an old haustorium showing a thickened inner region of the sheath and little or no middle or outer sheath (×18,500). fw = Fungal wall; ga = glandlike appendage of the sheath; gp = glycogen particles; HW = host cell wall; ir = inner region of the sheath; lb = lipid body; lm = laticifer matrix; mi = mitochondria; mr = middle region of the sheath; or = outer region of the sheath; sd = sheath debris; sh = sheath; sm = sheath membrane.

It is through the sheath that all material is exchanged between host and parasite, regardless of haustorial age; thus, the function of the sheath is more important in pathogenesis than its origin. It is difficult to visualize that such a dynamic area as the sheath around haustoria of *P. carthami* serves no particular function, as was suggested for the analogous structure around haustoria of *E. cichoracearum* (19). As pointed out by Ehrlich & Ehrlich (8), the rust and powdery mildew fungi are considered analogous in their mode of parasitism; however, the sheaths of the two groups may bear little resemblance.

The mechanism by which obligately parasitic fungi take nourishment from the host cell is not likely to be extremely variable. The physiological requirements may be different between fungus species and biotypes within species. Membrane-limited secretory bodies and secretory vesicles have been suggested as mechanisms involved in the uptake of materials into haustoria (3, 22). Ehrlich & Ehrlich (8) suggested a process analogous to pinocytosis in animal cells to account for the uptake of nourishment into the haustorium. Although we observed numerous vesicles within the cytoplasm of the host cells and glandlike appendages of the sheath (Fig. 10), it was difficult to determine if these vesicles developed within the cytoplasm, or whether they arose at the boundary of the encapsulation around the glandlike appendages. Ehrlich et al. (11) believed that vesicles may be of three origins; some may be cross sections of tubular host endoplasmic reticulum (ER); others may originate from host Golgi apparatus; and others may arise from the sheath boundary. Regardless of their origin, it was equally difficult to determine whether they actually function in the transporting of material to the sheath, or whether they result from host response to infection and are functionless. Such a functional apparatus as was proposed by Peyton & Bowen (22) and Berlin & Bowen (3) would explain the bits of "debris" that we observed in the sheath area of *P. carthami* (Fig. 10). It is doubtful that the entire process of nutrient uptake as proposed for *P. graminis* (8) is operative in *P. carthami,* since discrete channels (plasmodesmata ?) were not observed connecting the sheath matrix to the haustorial cytoplasm. We observed an increase in the amount of host ER at the haustorial boundary. This suggests a possible structural basis for the transport of matter to and from the haustorium, as proposed by Ehrlich et al. (11).

Host parasite interaction.—Since all material exchanged between host and parasite must pass through the sheath, it is not surprising that the sheath as suggested by Ehrlich & Ehrlich (8) plays a role in resistance and susceptibility or avirulence and virulence. The fine structure of the haustorium complex in the invaded perivascular cells of Nebraska 1-1-5 (resistant) and Nebraska 8 (susceptible) are clearly paralleled in early developmental stages (Fig. 12, 13). Although sheaths develop around haustoria in the incompatible host, they are more amorphic than sheaths around haustoria in the compatible host and lack the bits of debris. Soon after the haustorium becomes mature in perivascular cells of Nebraska 1-1-5, as evidenced by the expansion of the haustorial head and the development of a sheath, the cytoplasm of the invaded cell undergoes a general collapse. The collapse is not unlike that which occurs 12-18 days after infection of Nebraska 8 cells. The collapse of the host cytoplasm is accompanied by or immediately followed by a general deterioration of the sheath matrix. This appears to isolate the haustorium from any contact with the host plasma membrane (Fig. 14). Collapse of the sheath which isolates the haustorium is followed by a collapse of the cytoplasmic organelles of the haustorium (Fig. 15). This collapse does not occur simultaneously with the collapse of the host cells cytoplasm; and it is not uncommon to find haustoria in cells where the cytoplasm has completely collapsed. I found no evidence that the sheath persists after death of the haustoria, as reported by Ehrlich & Ehrlich (7).

Glycogen, the normal carbohydrate storage product in fungi, is abundant in haustoria and hyphae in the perivascular region of Nebraska 8 (Fig. 1, 7, 8, 10). Glycogen particles were not observed in haustoria or hyphae in the perivascular region of Nebraska 1-1-5 hypocotyls up to the time of haustorial collapse (Fig. 13, 14, 15). This absence of glycogen suggests that successful pathogenesis was curtailed before the haustoria received sufficient nutrients from the host cytoplasm to synthesize glycogen.

Although the sheaths around haustoria in Nebraska 1-1-5 perivascular cells degenerate, this probably does not manifest resistance. Resistance instead appears to result from the incompatibility of the host cell to the invading haustoria. Degeneration of the host cytoplasm precludes movement of material into the sheath. The material within the sheath may disappear as it is digested and absorbed into the haustorium, or it may degenerate when the enveloping cytoplasm collapses.

Possible nature of resistance.—Single membrane-bounded crystal-containing bodies (CCBs) were observed in the cells of the perivascular region of both Nebraska 1-1-5 and Nebraska 8 (Fig. 16, 17). They were more abundant in Nebraska 1-1-5. They were rarely observed in the outer cortical area. Such bodies have received considerable attention in the literature. They have been observed in a wide variety of plants and are thoroughly reviewed by O'Brien & Thimann (21) and Frederick et al. (13). They may be analogous to animal lysosomes or plant microbodies (6, 21). The functional role of the CCBs is speculative, although it has been suggested that they represent the storage sites of hydrolytic enzymes which are released for localized autolysis (6, 13, 21). The presence of CCBs has frequently been associated with degenerate plant tissue (6, 13, 27). Armentrout & Wilson (1) reported spherosomes (microbodies) to be actively involved in the process of parasitism of *Piptocephalis virginiana* on *Mycotypha microspora.* They observed that the microbodies of the host broke down soon after the host cell was penetrated by haustoria. They concluded that these bodies may be involved in autolysis of the host cell. Matile et al. (18) discovered several hydrolytic enzymes associated with microbodies, and proposed plant microbodies to be analogous to animal lysosomes in having autolytic properties.

The crystalline-containing bodies in the perivascular

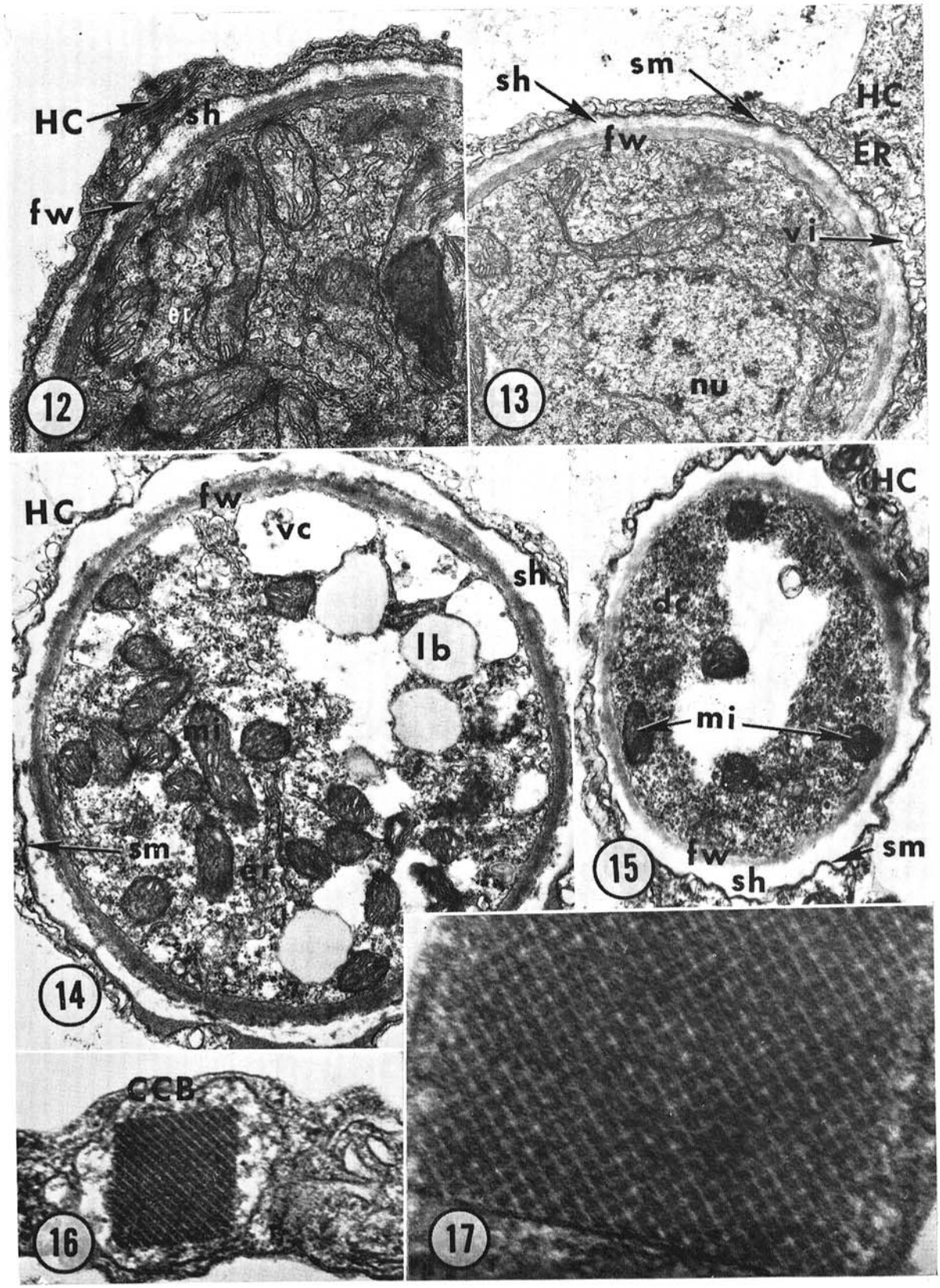
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Fig. 12-17. 12, 13) Sections through young haustoria of comparable age in perivascular cells of Nebraska 8, a susceptible line (12) and Nebraska 1-1-5, a resistant line (13). At this stage of haustorial development there is little ultrastructural difference in the haustoria in the two hosts (×25,500). **14)** A section through a haustorium in a perivascular cell of Nebraska 1-1-5 which is comparable to a haustorium of about equal age in a cell of Nebraska 8 shown in Fig. 10. The sheath has degenerated, leaving the haustorial wall isolated from contact with the invaginated host's plasma membrane. Glycogen is absent and some cytoplasmic degeneration is evident (×25,500). **15)** A haustorium in a perivascular cell of the incompatible line Nebraska 1-1-5. The haustorial cytoplasm shows advanced stages of degeneration, the sheath has completely degenerated, and the host-cytoplasm has collapsed (×18,500). **16, 17)** Cross sections of crystal-containing bodies which predominate in the perivascular region of the resistant variety and disappear when the cells containing them are invaded by haustoria. They may reflect some aspect of the resistance mechanism (×60,000 and 120,000). CCB = crystal-containing bodies; dc = degenerated fungal cytoplasm; ER = host endoplasmic reticulum; er = fungus endoplasmic reticulum; fw = fungal wall; HC = host cytoplasm; lb = lipid body; mi = mitochondria; nu = nucleus; vc = vacuole; sh = sheath; vi = vesicles in the host cytoplasm; sm = sheath membrane.

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cells of the safflower line Nebraska 1-1-5 readily disappear when cells are parasitized by haustoria. Solely on the basis of electron microscopical observations, it is tempting to suggest that the exclusionary resistance of Nebraska 1-1-5 may be associated with the predominance and rapid breakdown of the CCBs. The perivascular region where the CCBs and resistance factor are located is a degenerate region. The premature release of the material contained within the CCBs by the action of the fungus could cause autolysis of the host's cytoplasm and the destruction of the haustorium leading to resistance. A quantitative difference in the amount of autolytic enzyme released, as indicated by the lower frequency of CCBs in the perivascular cells, could explain the failure of the cells of Nebraska 8 to respond as those of Nebraska 1-1-5.

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