Haustoria and Intracellular Hyphae in the Rusts

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ABSTRACT

Prior to cell penetration by *Hemileia vastatrix*, a thick electron-opaque layer is formed in the haustorial mother cell against the wall in contact with a host cell. A thin, less dense zone is subsequently laid down over this layer. Coffee leaf cells respond to the presence of a haustorial penetration tube by producing a large host collar that reacts weakly, if at all, to callose-specific staining. Callose apposition onto the body of the haustorium appears to be linked to incipient necrotization, since only haustoria in senescent cells stain callose-positively. These haustoria have thick sheaths, whereas haustoria associated with cells of normal appearance are thin-sheathed and react negatively for callose. In the pycnial intercellular hyphae of *Puccinia sorghi*, no prepenetration layers are apposed against the wall in contact with a host cell. Penetration is apparently partly enzymatic, and the middle and inner layer of the wall are continuous with the innermost layers of intracellular fungal structures. The latter are filamentous, often septate, and may coil extensively around host organelles. Penetration tubes, neckbands, and haustoriumlike dilations are absent. A dearth of intracellular fungal structures, relative to a well-developed intercellular thallus, suggests that efficient use is made of nutrients diffusing from host cells. Preliminary studies on one other pycnial and three aecial stages indicate that these characteristics may well be typical of many pycnial and aecial stages. On the basis of the presented evidence, it is proposed that intracellular structures of this type be designated "intracellular hyphae" rather than "haustoria".

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Additional key words: rust, ultrastructure.

Light microscopic studies have indicated that haustoria of uredial and telial stages of rusts differ structurally from those of pycnial and aecial stages. Rice (22), for example, described haustoria of the uredial and telial stage as saccate to extensively lobed, and those of the pycnial and aecial stage as generally coiled and filamentous.

Most ultrastructural studies of rust haustoria have thus far been restricted to the uredial stage. The uredial haustorium is described and illustrated as an intracellular saccate or lobed organ, connected by a narrow penetration tube to an extracellular haustorial mother cell (4, 7, 9, 11, 12, 17). However, details concerning the ultrastructural nature of intracellular structures associated with aecial and pycnial rust stages are lacking and the present investigation was, therefore, undertaken to compare, by means of electron microscopy, intracellular structures formed by monokaryotic and dikaryotic mycelia.

MATERIALS AND METHODS.—The two host-rust associations selected for intensive studies were the pycnial stage of *Puccinia sorghi* Schw. on inoculated *Oxalis corniculata* L., and the uredial stage of *Hemileia vastatrix* Berk. & Br. on *Coffea arabica* L. Other readily available rusts examined were unidentified aecial stages on *Calendula officinalis* L. and *Senecio madagascariensis* Poir., the aecial stage of *P. sorghi* on *O. corniculata*, and a pycnial stage recently reported (27) on *Antirrhinum majus* L. Material was processed at the first sign of sporulation on young, fully expanded leaves of plants growing in a greenhouse at 20-26 C day and 17-20 C night temperatures.

Infected tissue was fixed for 6 hr with 6% glutaraldehyde in 0.05 M Na-cacodylate buffer at pH 7.2-7.4, postfixed for 2 hr with 2% osmium tetroxide in 0.05 M Na-cacodylate buffer at 7.2-7.4, dehydrated in an alcohol series, transferred to propylene oxide, and embedded in Araldite. Sections, cut with glass knives on a Porter-Blum MT-1, were stained for 50 min with 2% aqueous uranyl acetate, for 20 min with undiluted Reynolds' lead citrate, and examined with a Hitachi HU-11E.

Callose staining for light microscopy was effected with resorcinol blue (14) and fluorescent aniline blue (5).

RESULTS.—*Uredial stage of H. vastatrix*.—Intercellular fungal cells, including haustorial mother cells, are bounded by a triplex wall (Fig. 1), the middle layer being more electron-opaque than the inner and outer. The presence of a distinct layer between two hyphae, or between hyphal and host cells (Fig. 1 [see open arrows]), suggests nonspecific intercellular bonding. In the young binucleate haustorial mother cell a thick electron-opaque layer is apposed against the wall in contact with the host cell (Fig. 1). An early stage in the development of a thin, less dense zone, constituted over the electron-opaque layer, is also visible (Fig. 1 [see solid arrows]). Continuity between any of the deposited or pre-existent layers and the wall of the penetration tube could not unequivocally be demonstrated.

Coffee leaf cells respond to the presence of a haustorial penetration tube by producing a voluminous collar (Fig. 2). Immediately beyond the neckband, situated at or near the point of emergence from the collar, the penetration tube swells to form

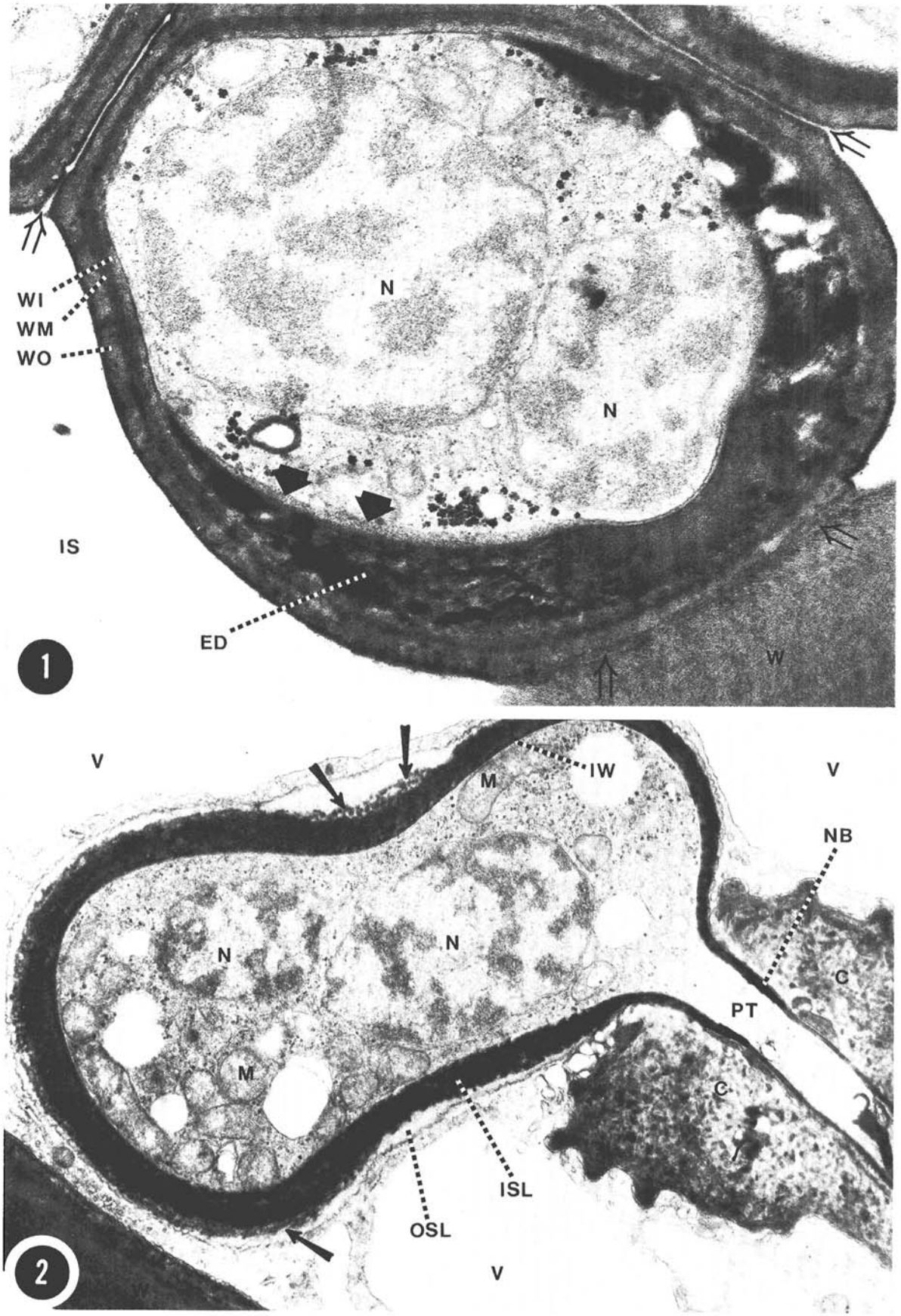

Fig. 1-2. *Hemileia vastatrix*. **1)** Localized electron-dense layer (ED) and early stage in formation of inner, less dense zone (solid arrows) in young haustorial mother cell. Wall of haustorial mother cell is composed of inner (WI), middle (WM), and outer (WO) layer. Abutting cells are separated by substance that is possibly adhesive (open arrows) (× 31,000). **2)** Penetration tube (PT) with collar (C) and neckband (NB) subtending haustorial body. Note thin wall (IW) of haustorium and merging (arrows) of inner (ISL) and outer (OSL) sheath layer (× 20,000). IS = intercellular space; M = mitochondrion; N = nucleus; V = vacuole; W = host cell wall.

the body of the haustorium (Fig. 2). The binucleate saccate haustorium is bounded by a thin wall, similar to the penetration tube wall, and a sheath consisting of an electron-dense inner layer that in places appears to merge (Fig. 2 [see arrows]) with the electron-lucent outer layer. We observed that thick haustorial sheaths, found in degenerating cells only, stain deeply with callose-specific stains, whereas collars react weakly if at all. The majority of haustoria, however, are thin-sheathed, associated with cells of normal appearance, and are negative in reaction.

Pycnial stage of P. sorghi.–The wall of intercellular hyphae is three-layered (Fig. 3). The outer layer is reflexed where it abuts on other surfaces, possibly attaching hyphal cells to the host and to other hyphae. At penetration, no specialized layers are apposed internally against the wall in contact with a host cell (Fig. 4). Host wall degradation around the penetrating hypha (Fig. 5 [see arrows]) indicates that penetration is apparently partly enzymatic. The wall of the intercellular mycelium, with the exception of the outer layer, is continuous with the wall of the intracellular fungal structure (Fig. 4, 6, 7). Typically, there is no pronounced reduction in the diameter of a penetrating hypha at the point of ingress, and neither a penetration tube nor a neckband is present (Fig. 6, 7). The hypha invaginates the host plasmalemma, becomes septate (Fig. 8), and may coil extensively around host organelles (Fig. 9). The two layers of fungal origin are enveloped by a duplex layer (Fig. 10) in which no distinction between a collar and sheath can be made (Fig. 6, 7). The collar/sheath consists of a granular inner and an electron-lucent outer zone (Fig. 10).

DISCUSSION.–Triplex intercellular hyphal walls have previously been found in *Melampsora larici-populina* (24), *H. vastatrix, Uromyces phaseoli*, and the pycnial stage of *P. sorghi* (25). The outer layer possibly has adhesive properties (9, 25). A layer, perhaps of a mucilagenous cementing nature, has also been observed between the haustorial mother cell wall and the host cell wall in wheat infected with *Puccinia graminis* f. sp. *tritici* (7).

Considerable thickening of the rust haustorial mother cell wall immediately adjacent to the penetration pore has been described and/or shown in micrographs by several authors (7, 9, 12, 16, 17, 18, 25, 26). Previous investigations (17, 25) and the results of the present study show that such thickening is due to localized deposition against the inner wall. Littlefield & Bracker (17) observed a continuity between the less dense zone, laid down over the localized opaque layer in haustorial mother cells of *M. lini*, and the fungal wall of the penetration tube. Such continuity was also apparent in several micrographs published earlier (4, 9, 12, 16).

It is evident from electron micrographs of *Erysiphe graminis* f. sp. *hordei* (2, 6, 21) that cell penetration by this fungus is similarly accompanied by deposition in the haustorial mother cell against the wall in contact with the host. The deposited layer is continuous with the wall of the penetration tube. Localized deposition is also characteristic of germ tube initiation by spores of *Fusarium culmorum* (19) and bud initiation of *Rhodotorula glutinis* (20). The presence of localized apposition in such diverse structures as rust and powdery mildew haustorial mother cells, fusarial spores, and yeast cells, suggests that a well-established mechanism of specialized hyphal emergence exists in Ascomycetes and Basidiomycetes.

The large host collar surrounding the penetration tube of *H. vastatrix*, contrary to evidence adduced by Hardwick et al. (9) in respect of *U. appendiculatus*, reacts weakly, if at all, to callose-specific resorcinol blue and fluorescent aniline blue stains. Thick-sheathed haustoria of *H. vastatrix* react in a callose-positive manner to both staining procedures, but the majority of haustoria are thin-sheathed and give a negative reaction. Recent histological studies on cowpea leaves, immune to rust infection, suggested that the main body of the haustorial sheath is composed of a calloselike material, but the collar, in a few instances, did not react in a callose-positive manner (10). Since in the coffee-*H. vastatrix* relationship (i) gradation in reaction type is virtually absent and (ii) there appears to be a positive correlation between haustorial stainability and degeneracy of cells invaded by them, we consider that callose apposition is associated with incipient necrotization.

Intracellular structures formed by pycnial and aecial stages of *P. sorghi* on *O. corniculata* are found in epidermal cells, as a result of direct host penetration by germinating basidiospores (1, 23), and in mesophyll cells, arising from intercellular hyphae (23). At the light microscope level, such structures in mesophyll cells of *O. corniculata* were described as abundant, arising from a short slender entrance stalk, frequently possessing more than one nucleus, evidently invaginating the host cytoplasm, rarely sheathed, branched, and much coiled – often around the host nucleus (23).

Our results support and supplement preliminary ultrastructural findings concerning intercellular hyphae and intracellular proliferations of the *P. sorghi* pycnial stage (25). The intercellular mycelium, apparently partly by enzymatic degradation, enters leaf cells where it becomes surrounded by a duplex collar/sheath layer. It is evident that intracellular proliferations and their initiation differ in several important respects when those of pycnial and uredial thalli are compared: (i) Prepenetration layers are not formed in the pycnial intercellular hypha against the wall in contact with a host cell, and the inner wall layers of the intercellular hypha are continuous through the host cell wall with the wall of the penetrating hypha. In the uredial haustorial mother cell the specialized inner zone, formed prior to penetration, becomes continuous with the fungal wall of the penetration tube. (ii) Penetrating hyphae do not constrict into narrow penetration tubes, although these were purported to be present in the pycnial stage of *P. sorghi* (23), nor can neckbands be

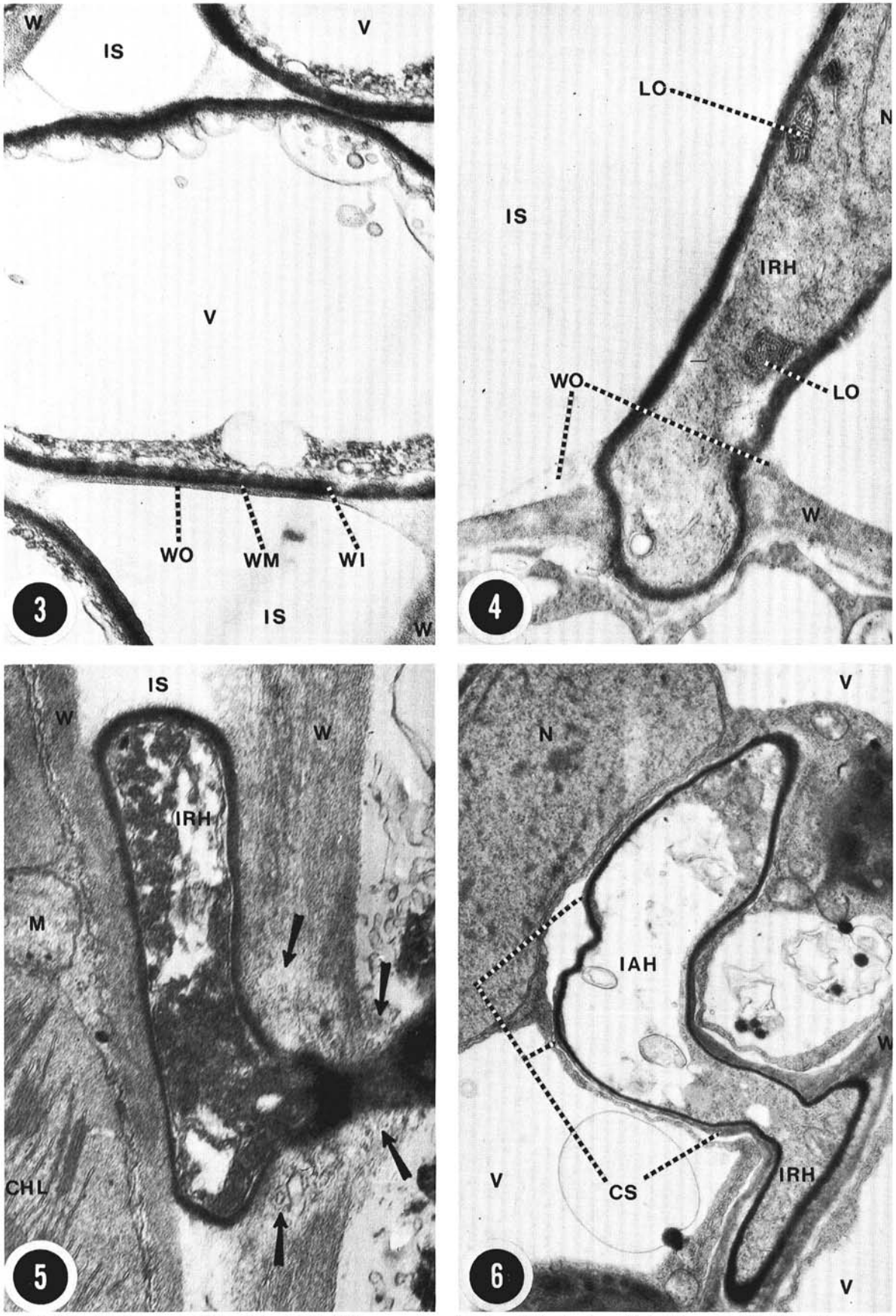

Fig. 3-6. Pycnial stage of *Puccinia sorghi.* **3)** Cross section of intercellular hypha with triplex wall (WI, WM, WO). Outer wall layer (WO) is reflexed where it abuts on other surfaces (× 37,000). **4)** Early stage in host cell penetration by intercellular hypha (IRH) (× 37,000). **5)** Degradation (arrows), apparently enzyme-induced, of host wall by penetrating hypha (IRH). Apparent constriction of hypha is due to glancing plane of section (× 35,000). **6)** Micrograph showing wall continuity between intercellular (IRH) and intracellular (IAH) hypha. Note collar/sheath (CS) and absence of neckband (× 11,000). CHL = chloroplast; IS = intercellular space; LO = lomasome; M = mitochondrion; N = nucleus; V = vacuole; W = host cell wall.

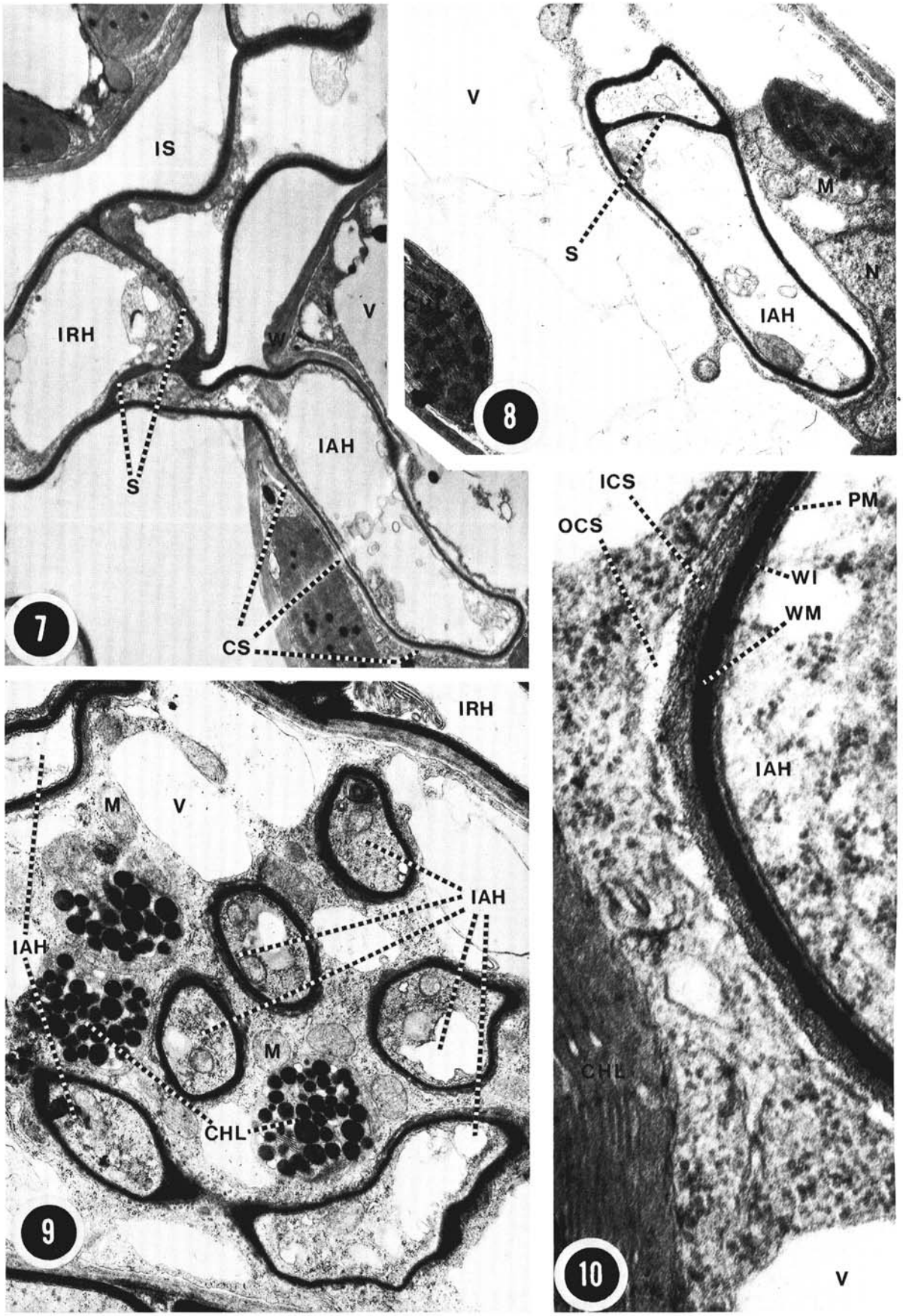

Fig. 7-10. Pycnial stage of *Puccinia sorghi*. 7) Micrograph showing interrelationship between intercellular (IRH) and intracellular (IAH) hypha. Note collar/sheath (CS) and absence of prepenetration layers and neckband (× 10,500). 8) Intracellular septate (S) hypha (IAH) (× 12,000). 9) Cross section of coiled intracellular hypha (IAH) (× 14,900). 10) Intracellular hypha (IAH), showing two fungal wall layers (WM, WI) enveloped by electron-dense inner (ICS) and electron-lucent outer (OCS) collar/sheath layer (× 88,000). CHL = chloroplast; M = mitochondrion; N = nucleus; PM = plasmalemma; S = septum; V = vacuole; W = host cell wall.

distinguished. (iii) Penetrating hyphae do not become saccate or lobed, as do uredial penetration tubes, but develop into filamentous, often septate structures that may coil extensively around host organelles.

Preliminary ultrastructural investigations of unidentified aecial rusts on *C. officinalis* and *S. madagascariensis*, the aecial stage of *P. sorghi* on *O. corniculata* and a pycnial stage on *A. majus*, showed that the type of cell penetration by, and intracellular growth of, the pycnial stage of *P. sorghi* may well be typical not only of many pycnial, but also aecial stages. Furthermore, each pycnial and aecial stage examined thus far has been characterized by a well-developed intercellular thallus and a relative dearth of intracellular structures. Evidently, these rust stages have either a very efficient mechanism of nutrient transfer from intracellular structures to intercellular hyphae or, more likely, an intercellular thallus that is able to subsist largely on substances diffusing from host cells.

In many respects, therefore, intracellular structures of the pycnial and aecial stages, examined during the course of this investigation, are strikingly different from haustoria of uredial stages. Since a haustorium is generally defined as a feeding organ which is markedly specialized in structure, the intracellular pycnial and aecial proliferations should be designated "intracellular hyphae" and not "haustoria". Generally, intracellular hyphae of other parasitic fungi do not appear to be surrounded by sheaths or collars (3, 13, 15), but a sheathlike structure around intracellular hyphae of *Phytophthora parasitica* f. sp. *nicotianae* in tobacco roots has been reported (8).

The relatively unspecialized growth habit of pycnial and aecial mycelia may point to their potential culturability on axenic media, and may explain the wide host range of some pycnial and aecial rusts in contrast to the extreme host-specificity of uredial and telial stages.

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