AN ULTRASTRUCTURAL STUDY ON RUSTS WITH SPECIAL REFERENCE TO PUCCINIA SORGHI ON OXALIS CORNICULATA

by

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A C K N O W L E D G E M E N T S

Indeed, from His fulness we have,
all of us, received . . . .
- John 1:16

CHAPTER 1

HAUSTORIA AND INTRACELLULAR HYPHAE*)

Introduction

Light microscopic studies have indicated that haustoria of uredial and telial stages of rusts differ structurally from those of pycnial and aecial stages. Rice (1927), 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 (Bracker & Littlefield, 1973; Coffey, Palevitz & Allen, 1972; Ehrlich & Ehrlich, 1971; Hardwick, Greenwood & Wood, 1971; Heath, 1972; Heath & Heath, 1971; Littlefield & Bracker, 1972). However, details concerning the ultrastructural nature of intracellular structures associated with aecial and pycnial rust stages are lacking and this part of the investigation was, therefore, undertaken to compare 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.,

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*) An article based on this chapter has appeared in Phytopathology 63: 281-286.

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 (Truter & Martin, 1971) 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 lead citrate (Reynolds, 1963), and examined with a Hitachi HU-11E.

Callose staining for light microscopy was effected with resorcinol blue (Johansen, 1940) and fluorescent aniline blue (Currier & Strugger, 1956).

Observations

Intercellular cells of the uredial stage of H. vastatrix, 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 - 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
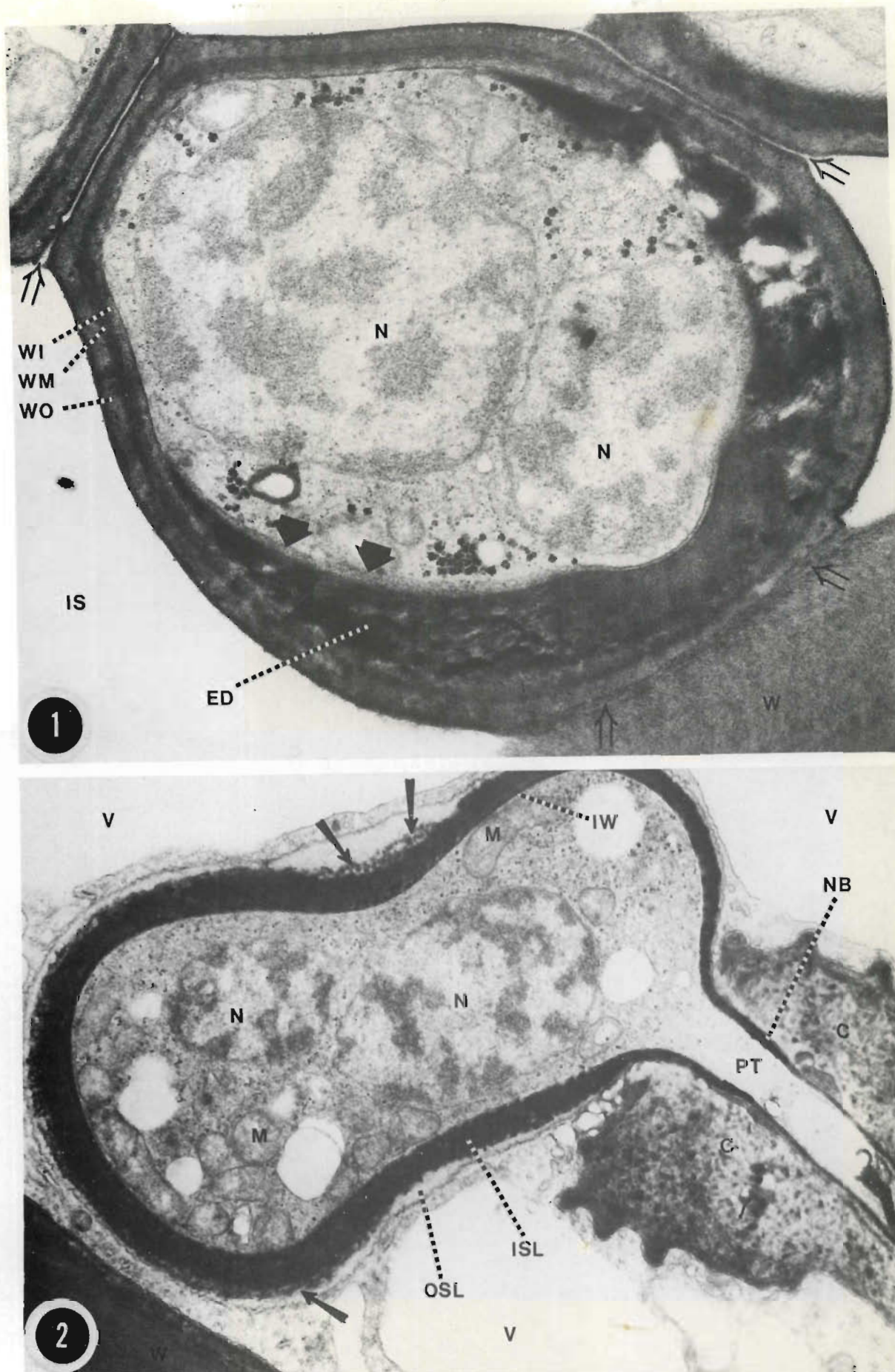
Fig. 1-2. *Hemileia vastatrix.* 1) Localized electron-dense layer (ED) and early stage in formation of inner, less dense zone (solid arrows) in young haustorial mother cell. Wall of haustorial mother cell is composed of inner (WI), middle (WM), and outer (WO) layer. Abutting cells are separated by substance 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.
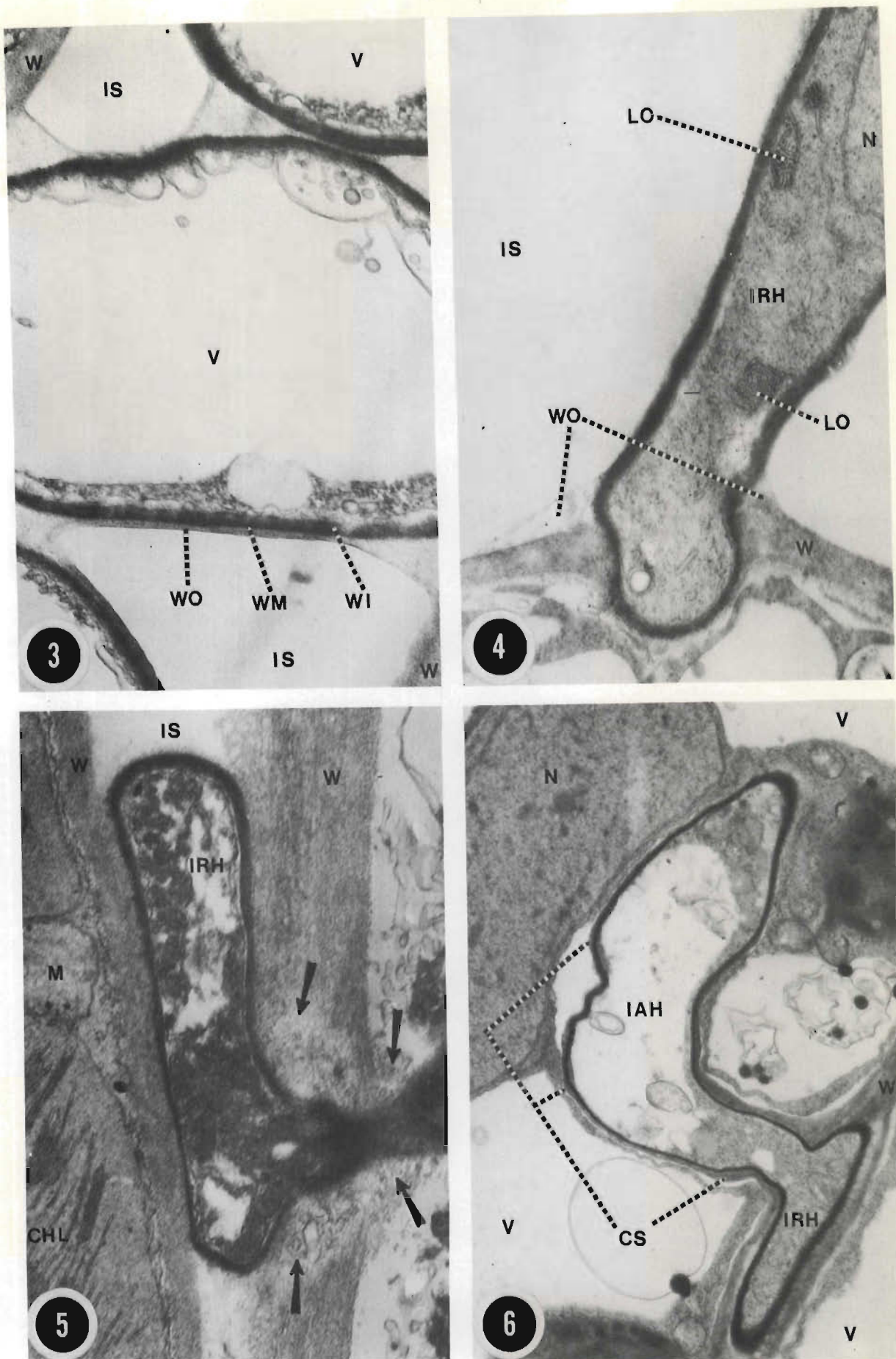
Fig. 3-6. Pycnial stage of *Puccinia sorghi.* 3) Cross section of intercellular hypha with triplex wall (WI, WM, WO). Outer wall layer (WO) is reflexed where it abuts on other surfaces (× 37,000). 4) Early stage in host cell penetration by intercellular hypha (IRH) (× 37,000). 5) Degradation (arrows), 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.
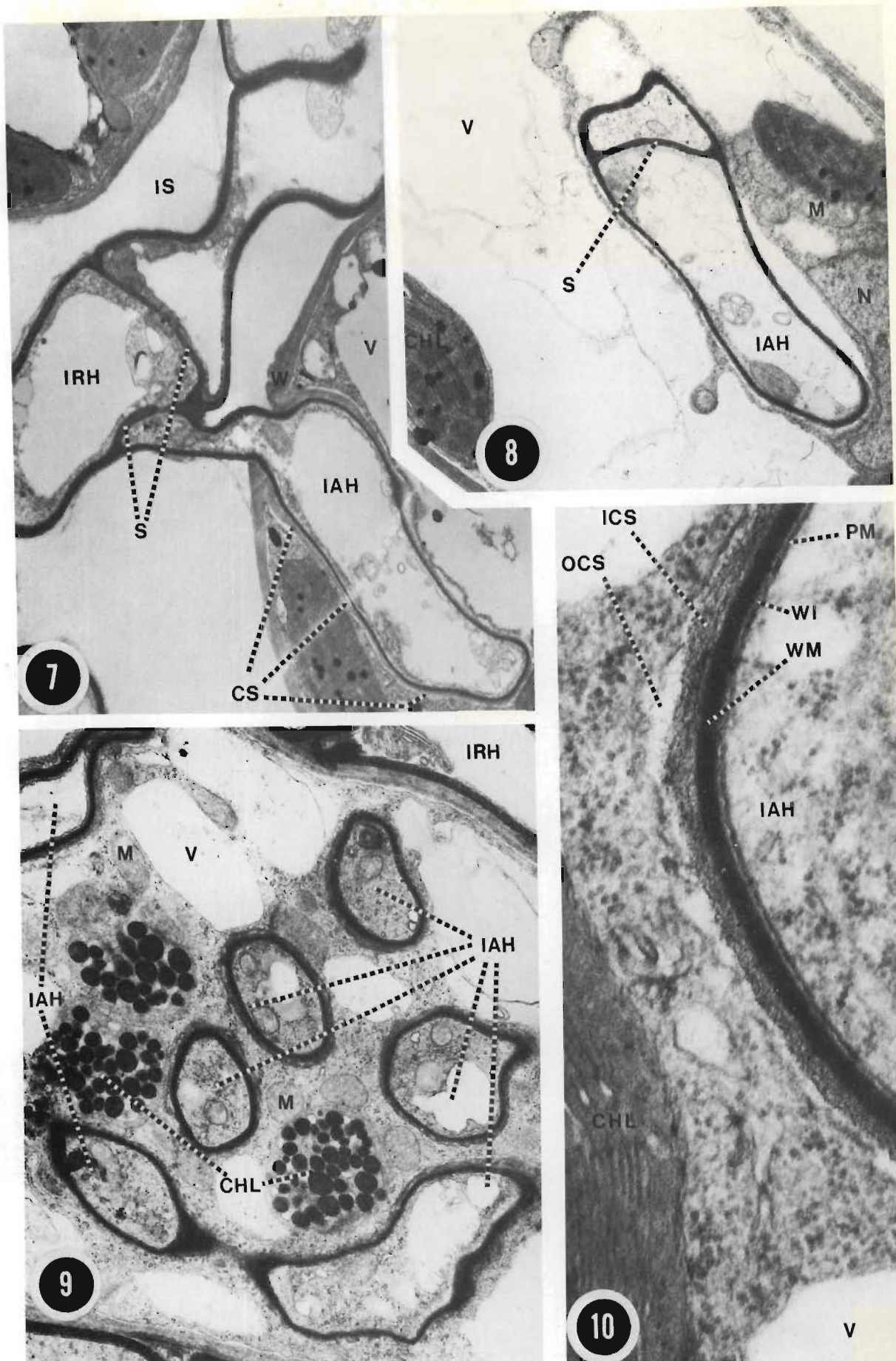
Fig. 7-10. Pycnial stage of *Puccinia sorghi*. 7) Micrograph showing interrelationship between intercellular (IRH) and intracellular (IAH) hypha. Note collar/sheath (CS), and absence of prepenetration layers and neckband (X 10,500). 8) Intracellular septate (S) hypha (IAH) (X 12,000). 9) Cross section of coiled intracellular hypha (IAH) (X 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 (X 88,000). CHL = chloroplast; M = mitochondrion; N = nucleus; PM = plasmalemma; S = septum; V = vacuole; W = host cell wall.

the development of a thin, less dense zone, constituted over the electron-opaque layer, is also visible (Fig. 1 - 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 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 and an electron-lucent outer layer. The two sheath layers in places appear to merge (Fig. 2 - arrows). It was found that thick haustorial sheaths, present 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.

The wall of intercellular hyphae of P. sorghi 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 - arrows) intimates that penetration is at least 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 (Figs. 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 (Figs. 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 (Figs. 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 (Rijkenberg, 1972), H. vastatrix, Uromyces phaseoli, and the pycnial stage of P. sorghi (Rijkenberg & Müller, 1971). The outer layer possibly has adhesive properties (Hardwick, Greenwood & Wood, 1971; Rijkenberg & Müller, 1971). 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 (Ehrlich & Ehrlich, 1971), and in other host - rust relationships (Bracker & Littlefield, 1973).

Considerable thickening of the rust haustorial mother cell wall immediately adjacent to the penetration pore has been described and/or shown in micro-graphs by several authors (Bracker & Littlefield, 1973; Ehrlich & Ehrlich, 1971; Hardwick et al., 1971; Heath & Heath, 1971; Littlefield & Bracker, 1970; Littlefield & Bracker, 1972; Manocha & Shaw, 1967; Rijkenberg & Müller, 1971; Shaw & Manocha, 1965). Other investigations (Bracker & Littlefield, 1973; Littlefield & Bracker, 1972; Rijkenberg & Müller,

1971) and the results of the present study show that such thickening is due to localized deposition against the inner wall. Littlefield & Bracker (1972) 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 (Coffey, Palevitz & Allen, 1972; Hardwick et al., 1971; Heath & Heath, 1971; Littlefield & Bracker, 1970), and Bracker & Littlefield (1973), in a comprehensive review, consider that claims for its absence in the penetration pore of rusts have not been substantiated by reliable ultrastructural evidence.

It is evident from electron micrographs of Erysiphe graminis f. sp. hordei (Bracker, 1968; Edwards & Allen, 1970; McKeen & Bhattacharya, 1970) 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 (Marchant, 1966) and bud initiation of Rhodotorula glutinis (Marchant & Smith, 1967). 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. (1971) 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 callose-like material, but the collar, in a few instances, did not react in a callose-positive manner (Heath, 1971). 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, it seems that callose apposition is associated with incipient necrotization. On the basis of the classification of host - symbiont interfaces presented by Bracker & Littlefield (1973), the interface between host cells and intracellular structures of H. vastatrix and P. sorghi, is of the common IT 24 type.

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 (Allen, 1934; Rice, 1933), and in mesophyll cells, arising from intercellular hyphae (Rice, 1933). 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 (Rice, 1933). Observations made during the course of the present investigation support and supplement preliminary ultrastructural findings concerning intercellular hyphae and intracellular proliferations of the P. sorghi pycnial stage (Rijkenberg & Müller, 1971). The intercellular mycelium,

apparently at least 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) Pre-penetration 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 (Rice, 1933), nor can neckbands be distinguished. (iii) Penetrating hyphae do not become saccate or lobed, as do uredial haustoria, 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 author proposes that the intracellular pycnial and aecial proliferations be designated "intracellular hyphae" and not "haustoria". Generally, intracellular hyphae of other parasitic fungi do not appear to be surrounded by sheaths or collars (Calonge, 1969; Hess, 1969; Kazama & Fuller, 1970), but a sheath-like structure around intracellular hyphae of Phytophthora parasitica f. sp. nicotianae in tobacco roots has been reported (Hanchey & Wheeler, 1971).

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.

CHAPTER 2
SPOROGENESIS IN THE PYCNIAL STAGE OF PUCCINIA SORGHI*)

Introduction

Detailed light microscopic studies of pycniospore ontogeny in Gymnosporangium clavariiforme Rees (Blackman, 1904) and G. clavipes Cooke & Peck (Olive, 1944) have been made. Blackman observed the formation of a septum between the pycniospore and its sporophore, and, at the distal end of the sporophore, noted a ring-like thickening that stained with Congo red. According to Olive, the young pycniospore is formed by budding and pushed through an open collar at the tip of the sporophore enlarging just above the rim of the collar. The mature pycniospore is then cut off by basal constriction and displaced by the succeeding pycniospore bud.

Hughes (1970), in an account of the ontogeny of spore forms in the Uredinales, expressed the opinion that, on the basis of the description by Olive (1944), the sporogenous cells in G. clavipes are clearly phialides and suggested that perhaps the sporogenous cells of all pycnia are phialides. In Hughes' account no mention was made of the description of pycniosporogenesis by Blackman (1904).

In this part of the study pycniosporogenesis of maize rust, Puccinia sorghi Schw., on the alternate host, Oxalis corniculata L., is described.

Materials and Methods

Leaf tissue of glasshouse-grown, artificially inoculated O. corniculata plants, bearing young erumpent pycnia, was fixed, dehydrated and embedded

as described in Chapter 1. Embedded material was sectioned with an LKB UM3 ultrotome, using a diamond knife. Sections were stained with 2% aqueous uranyl acetate for 50 min followed by undiluted lead citrate (Reynolds, 1963) for 20 min, and examined with a Hitachi HU-11E electron microscope.

Observations

The pycnium is bounded by a pseudoparenchymatous layer in which component cells are often vacuolate (Fig. 1) and possess three-layered walls (Fig. 2). The outer layer of the wall is thick and confluent with the outer layer of adjacent cell walls (Figs. 1, 2). Septal pores were sectioned infrequently in septa of the pycnium, but more commonly in inter- and intracellular hyphae. A pulley-wheel apparatus (Fig. 3) was evident in all median cross-sections of pores.

Pycniosporophores are unicellular, slightly tapered towards the apex and filled with densely staining cytoplasm (Fig. 1). Vacuoles, when present, are mainly situated in a proximal position (Fig. 1). Sporophores contain a single nucleolate nucleus (Fig. 1), many mitochondria (Fig. 8) and a few lipid bodies (Fig. 7).

After division of the sporophore nucleus, a daughter nucleus migrates into the spore primordium and a complete septum is laid down centripetally (Figs. 4, 6). The septum is composed of two electron-opaque walls separated by an electron-lucent lamella (Fig. 5). Against the periclinal wall the septal walls widen considerably, assuming a nearly triangular appearance in cross-section (Fig. 5). Wall deposition continues after septum formation,
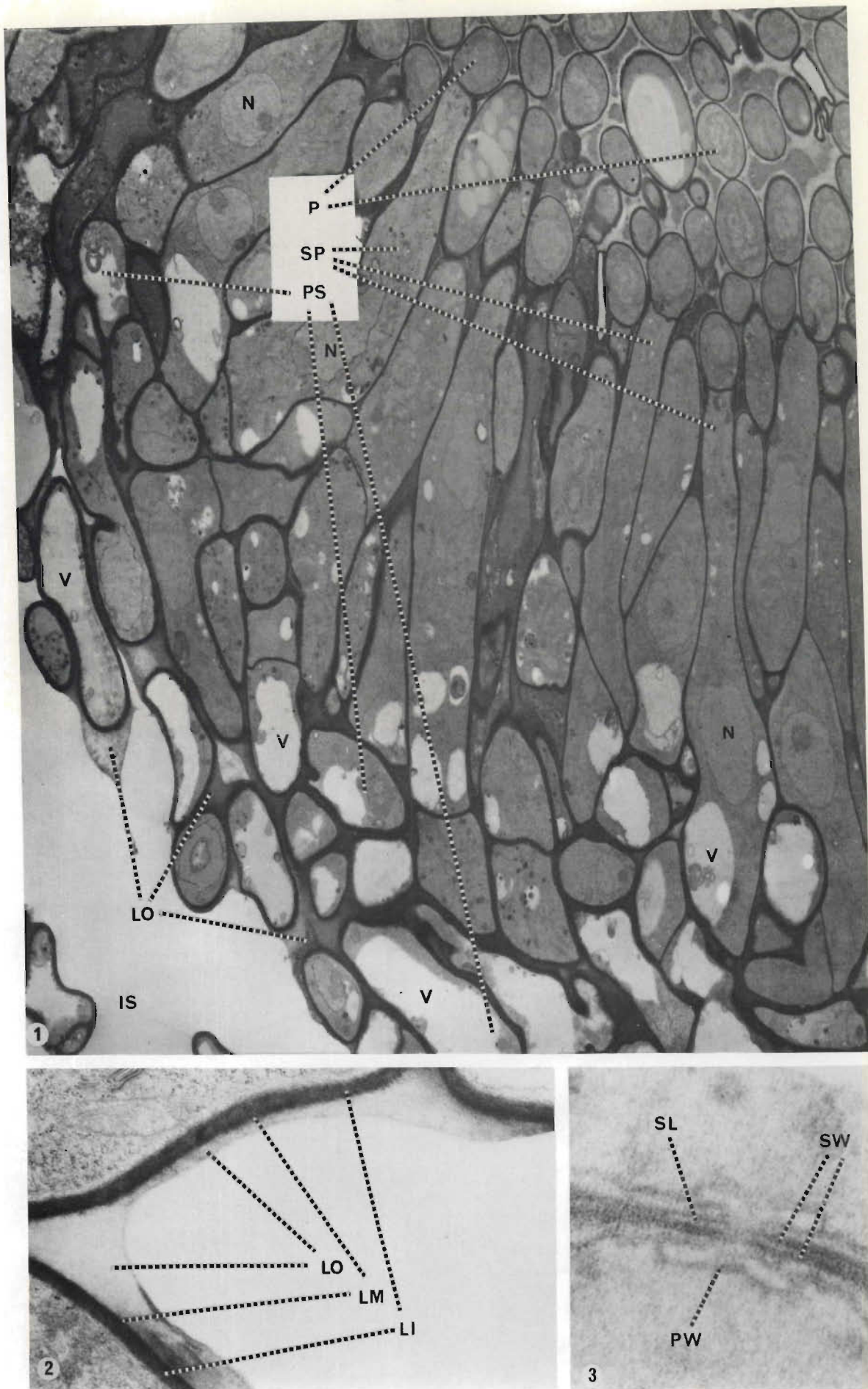
Figs. 1-3. 1) Section through spore-bearing region of pycnium. Note outer layer of pseudoparenchymatous cell wall (LO) and nucleolate nucleus (N) of sporophore (SP) (x 3,100). 2) Wall of pseudoparenchymatous cells showing inner (LI), middle (LM) and outer layer (LO) (x 27,000). 3) Pore with pulley-wheel apparatus (PW) between pseudoparenchymatous cell and pycniosporophore (x 120,000). IS = intercellular space; P = pycniospore; PS = pseudoparenchymatous cell; SL = septal lamella; SW = septal wall; V = vacuole.
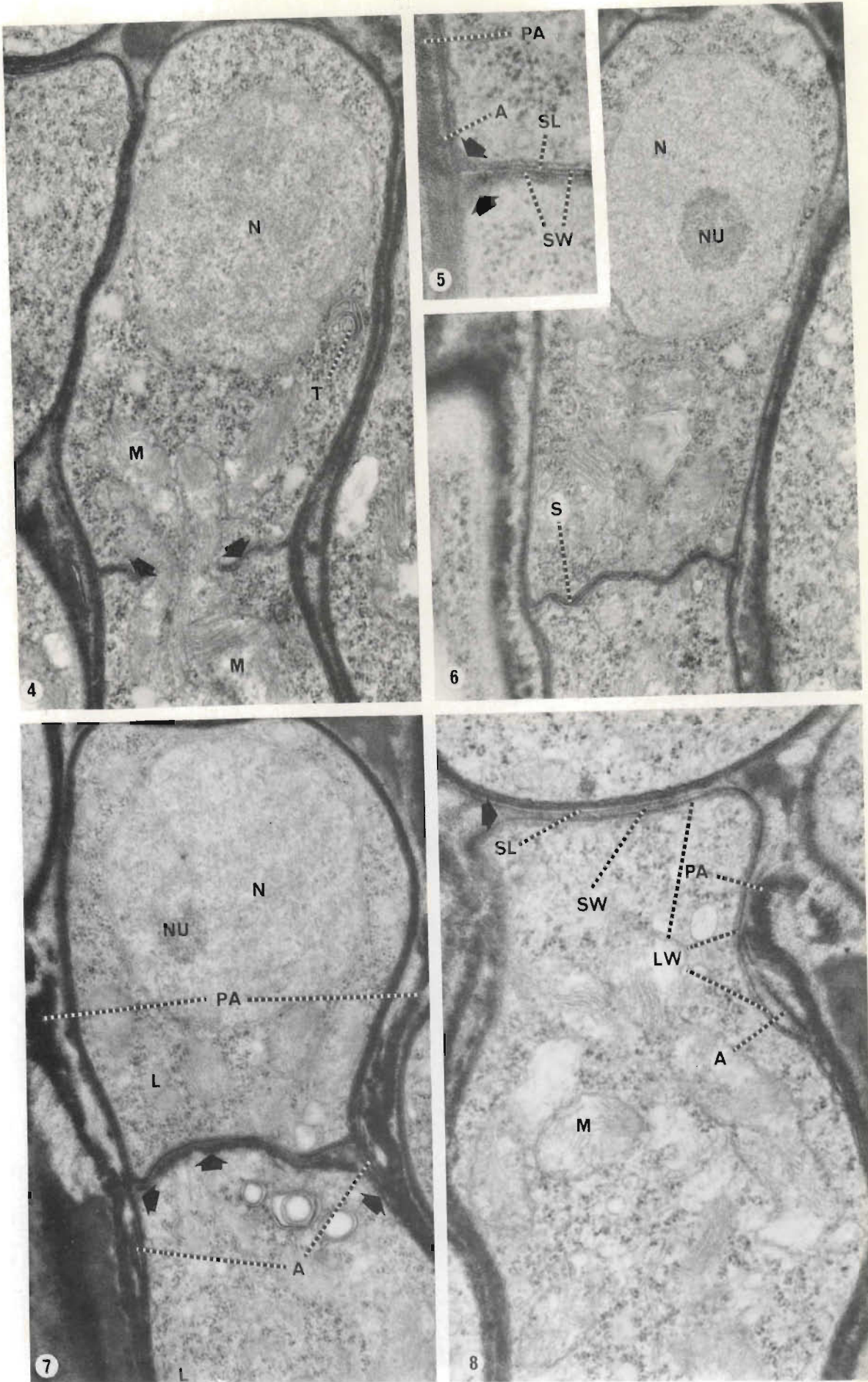
Figs. 4–8.   4) Centripetal formation of pycniospore – sporophore septum (arrows) (x 18,600).   5) Widening (arrows) of septal walls (SW) against periclinal wall (x 32,400).   6) Complete pycniospore – sporophore septum (S). Absence of annellation suggests this is primary pycniospore (x 17,900).   7) Early stage in deposition (arrows) of localised wall in sporophore tip. Note lipid bodies (L), annellations (A) and primary annellation (PA) (x 17,800).   8) Extension of localised wall (LW). Note (i) primary annellation (PA) and annellation (A) that has resulted from tearing of previous periclinal wall, and (ii) continued development of fracture (arrow) at lower septal wall (SW) (x 22,900). A = annellation; M = mitochondrion; N = nucleus; NU = nucleolus; PA = primary annellation; SL = septal lamella; T = tubule-containing structure.
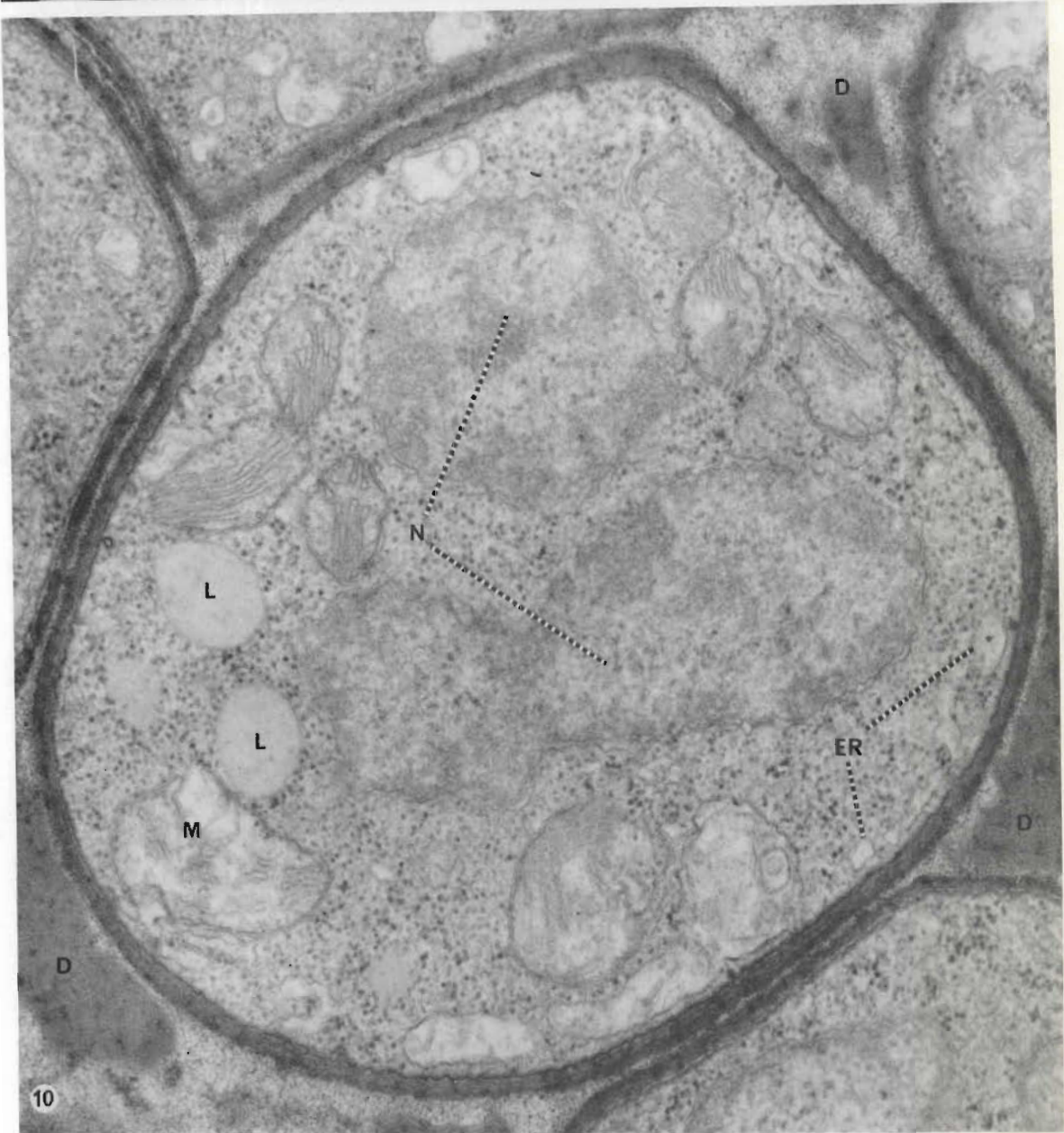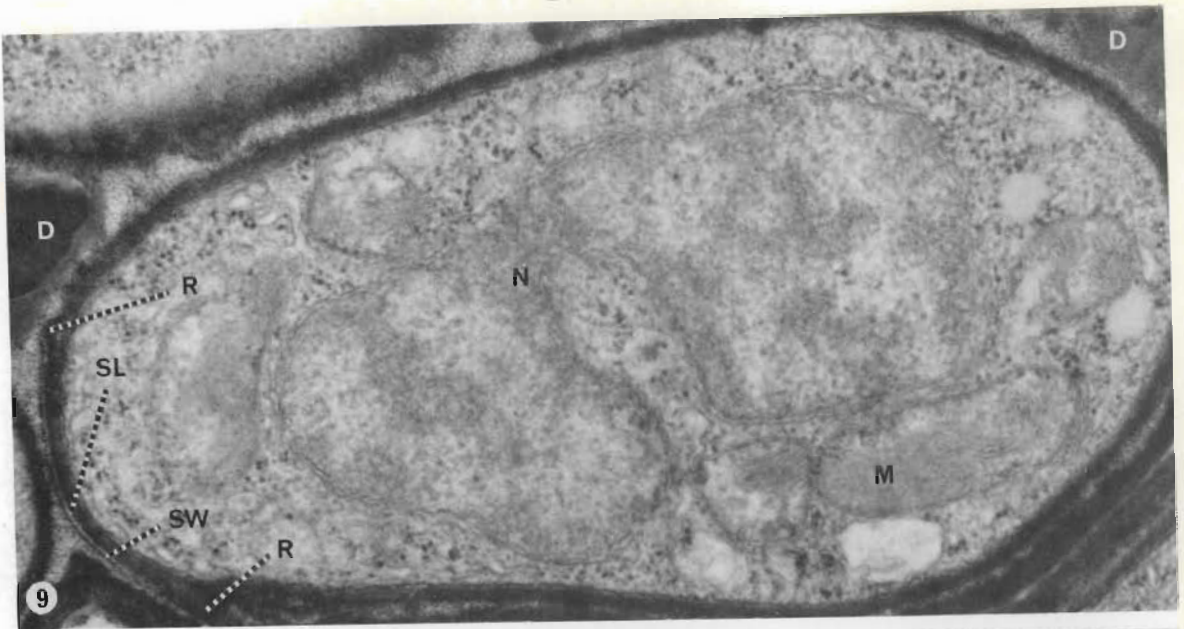
Figs. 9-10. 9) U-shaped nucleus (N) in pycniospore. Note debris (D), attached electron-opaque remnant of lower septal wall (SW), electron-lucent septal lamella (SL) and ridge-like frill (R) of hilum (x 23,200). 10) Section through arms of U-shaped pycniospore nucleus (N) (x 30,000).   ER = endoplasmic reticulum; L = lipid body; M = mitochondrion.

and a localised wall is laid down against the existing wall of the sporophore tip (Fig. 7). Spore release is effected by the development of an anticlinal fracture plane at the lower septal wall (Fig. 8) and the rupture of the periclinal wall at the septal periphery. The newly formed localised wall of the sporophore tip extends beyond the remnants, or annellation, of the periclinal wall, developing into a succeeding pycniospore primordium (Fig. 8). At the spore hilum the septal lamella, a remnant of the lower septal wall, and a poorly developed basal frill, possibly derived from the widened part of the septal walls, are retained (Fig. 9). After secession the spore wall increases in thickness from $\pm$ 45 nm to $\pm$ 85 nm.

The detachment of successive pycniospores gives rise to a series of annellations which are borne at approximately the same level on the sporophore (Fig. 7). The outer annellation results from the formation of the sporophore's primary spore and is the most substantial (Figs. 7, 8).

Pycniospores are released into the pycnial cavity where they lie closely packed (Fig. 1) in a matrix containing a considerable quantity of debris (Figs. 9, 10). The spores possess a single nucleus with a small nucleolus (Figs. 6, 7). In mature pycniospores the nucleus often assumes a U-shape (Fig. 9) and sectioned spores may, therefore, appear to contain two nuclei (Fig. 10). Spore mitochondria are short with predominantly plate-like cristae (Figs. 9, 10). Tubule-containing structures (Fig. 4), many ribosomes, some endoplasmic reticulum and a paucity of lipid bodies (Figs. 9, 10) characterize spore cytoplasm.

Discussion

The wall of pseudoparenchymatous cells in the pycnium, like that of inter-cellular hyphae (Chapter 1), is three-layered. The thick and confluent character of the outer wall layer of pseudoparenchymatous cells in the pycnium has also been observed in primordial cells of Uromyces appendiculatus uredia (Müller, Rijkenberg & Truter, 1974a). The function of such thick wall layers in rust fructifications is not known, but may relate to the water balance of these structures.

A pulley-wheel apparatus was observed in all median cross-sections of pores. It could not be determined if the scarcity of sectioned pores in pycnia indicates that, in addition to those between spore and sporophore, other septa in the pycnium are complete. If a pulley-wheel apparatus should also be present in septal pores of flexuous hyphae and their hyphal connections with aecial primordia, considerable modifications would be ne-cessary to allow nuclear migration.

In general, pycniospore formation and secession, as described here, does not differ significantly from the light microscopic observations of G. clavari-iforme made by Blackman (1904). The ontogeny of P. sorghi pycniospores, and possibly that of G. clavariiforme pycniospores, is annellophoric, suc-cessive spores on a sporophore seceding at approximately the same level, and does not differ markedly from annellophoric spore development in some Fungi Imperfecti (Sutton & Sandhu, 1969). However, the criterion that in annel-lophoric development the septum delimiting the conidium from the conidio-phore splits, so that the subsequent proliferation of the annellophore involves the exposed half septum (Hughes, 1971), is not fully met, since the septal

lamella and remnants of the lower septal wall remain attached to the hilum of a detached pycniospore. That Olive (1944) described a phialidic development of pycniospores in G. clavipes, can possibly be ascribed to limitations imposed by light microscopy. By referring to "... buds which enlarge to full size and are then separated by constriction", Arthur (1929), in a general description of pycniospore ontogeny, similarly implied phialidic development.

The "curious ring of thickening" (Blackman, 1904) and the "collar" (Olive, 1944) observed on pycniosporophores are, in all probability, annellation zones. When, as in Fig. 5, an annellation zone cannot be distinguished, the developing spore is probably the first spore formed in the basipetal sequence.

Olive (1944) considered that cell wall formation around the pycniospores of G. clavipes "appears to be a secondary phenomenon, associated with the transfer of the spermatium from a liquid medium to the air". In the present study it is shown that pycniospores of P. sorghi have a well-defined wall from their inception. Other characteristics of P. sorghi pycniospores include a small nucleolus in a commonly U-shaped nucleus, an appreciable volume of cytoplasm and short mitochondria with predominantly plate-like cristae. The inability of pycniospores to germinate may be related to the paucity of lipid bodies in these spores. Other spore stages in the Uredinales, viz. aeciospores (Walkinshaw, Hyde & Van Zandt, 1967), uredospores (Sussman, Lowry, Durkee & Maheshwari, 1969) and teliospores (Müller, Rijkenberg & Truter, 1974b) germinate well and contain many fat globules. No function can, as yet, be assigned to the tubule-containing structures commonly ob-

served in this pycnial stage, but reference to similar structures in the <u>P.</u>
<u>sorghi</u> aecial stage will be made in Chapter 3.

CHAPTER 3

THE AECIAL STAGE OF

PUCCINIA SORGHI

Introduction

Several comprehensive light microscopic investigations have been made of the aecial stage of maize rust, Puccinia sorghi Schw., on the alternate hosts, Oxalis spp. (Allen, 1934; Rice, 1933; Savile, 1939), but no ultrastructural studies of this stage have thus far been published.

The purpose of this aspect of the work on P. sorghi was to examine the aecial stroma, the peridium and sporogenesis in the aecium.

Materials and Methods

Using a micropipette, pycnial exudate was collected from a number of infections on inoculated leaves of Oxalis corniculata L. and, after mixing, applied to pycnia of these infections to achieve fertilization. As confluent pycnia might have complicated the ultrastructural interpretation, only pycnia occurring singly on leaflets were fertilized in this experiment. The host plants were kept in a greenhouse at 20 - 26°C day and 17 - 20°C night temperatures. When young aecia became erumpent approximately 6 days later, infected tissue was fixed, dehydrated, embedded, sectioned, stained and examined as described in Chapter 2.

Observations

Walls of cells in the base of the plectenchymatous stroma (Fig. 1) are
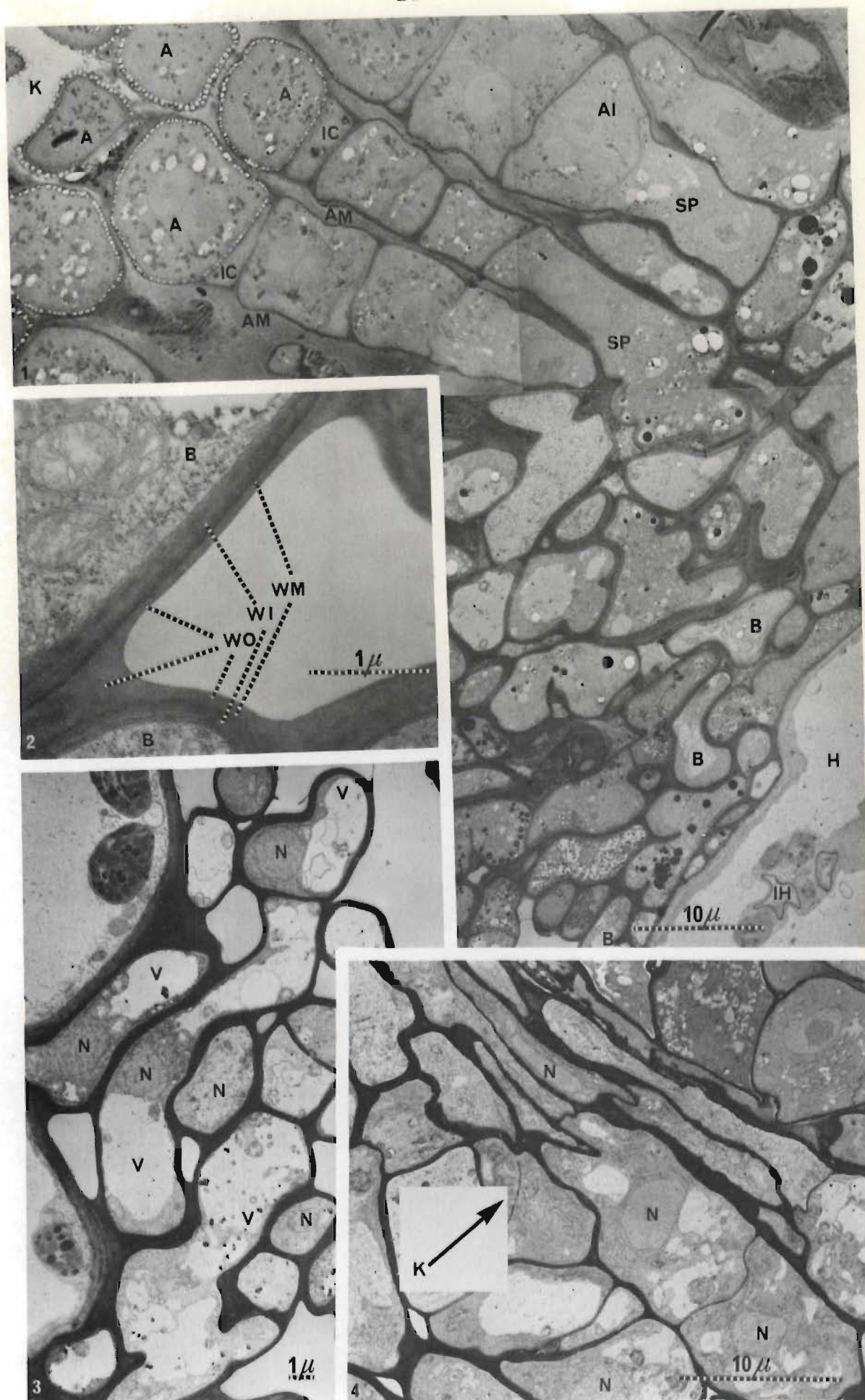
Figs. 1-4. 1) Section through aecium of Puccinia sorghi. Montage of three electron micrographs showing host cell (H), cells (B) of basal stroma, sporophores (SP), aeciospore initials (AI), intercalary cells (IC) and aeciospores (A). Host cell contains coiled intracellular hypha (IH) in cross-section. Note aecial matrix (AM) from which spores emerge into aecial cavity (K). 2) Parts of cells (B) of basal stroma showing inner layer (WI), middle layer (WM) and outer layer (WO) of wall. Outer layer is confluent between cells. 3) Section of cells in basal stroma, illustrating monokaryotic (N) and vacuolate (V) condition of the cell.
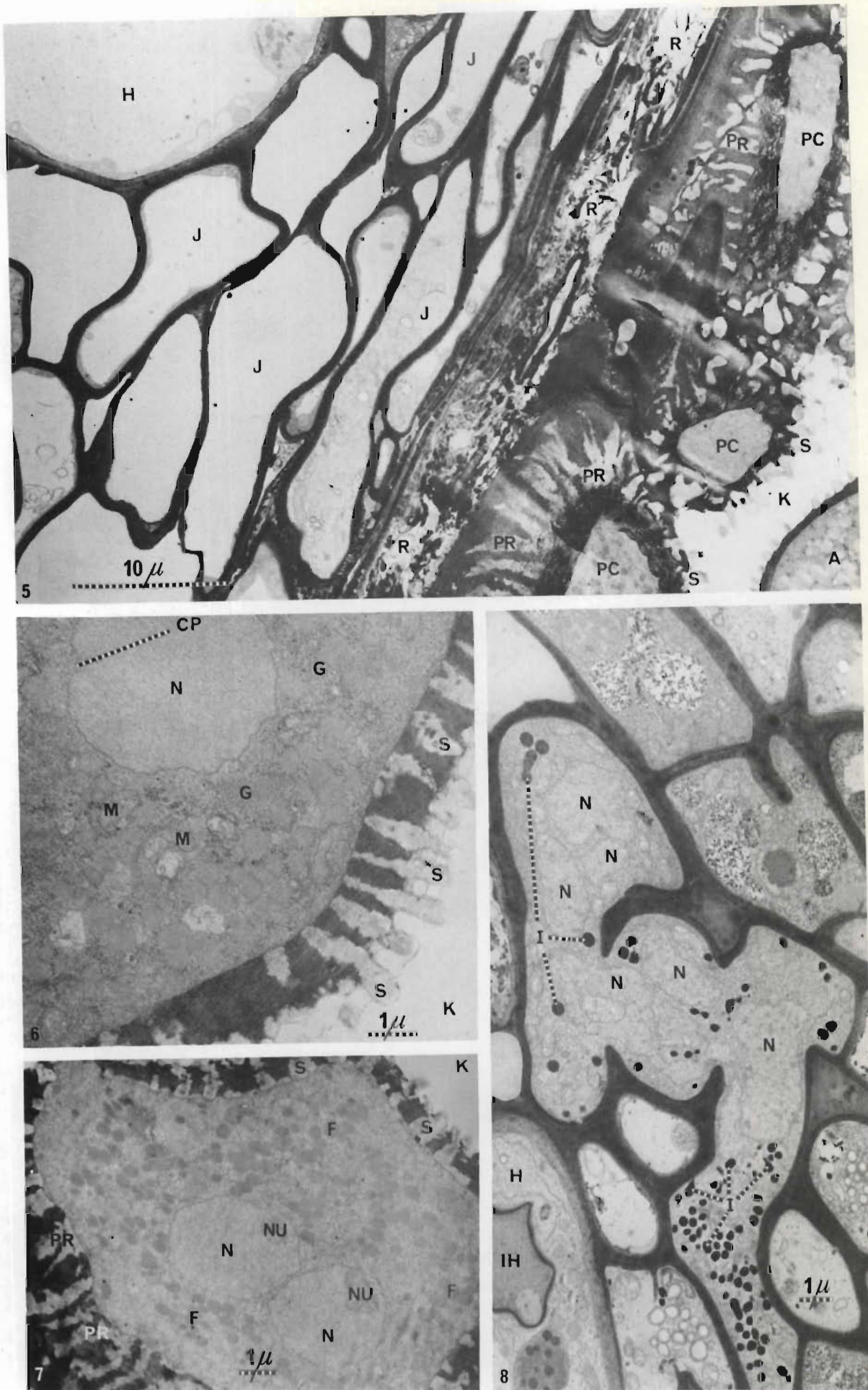
Figs. 5-8. 5) Micrograph showing relationship of host cell (H) to vacuolate prosenchymatous cells (J), layer of compressed cell residue (R), obliquely sectioned layer of peridial cells (PC) and aecial cavity (K). Note spines (S) and electron-transparent processes (PR) embedded in peridial cell walls. 6) Part of peridial cell bordering on aecial cavity. Note glycogen deposits (G), spines (S), short mitochondria (M) and spherical nucleus (N) with centriolar plaque (CP). 7) Section of peridial cell. Note spherical shape of nuclei (N), small nucleoli (NU), fat globules (F), spines (S) and electron-transparent
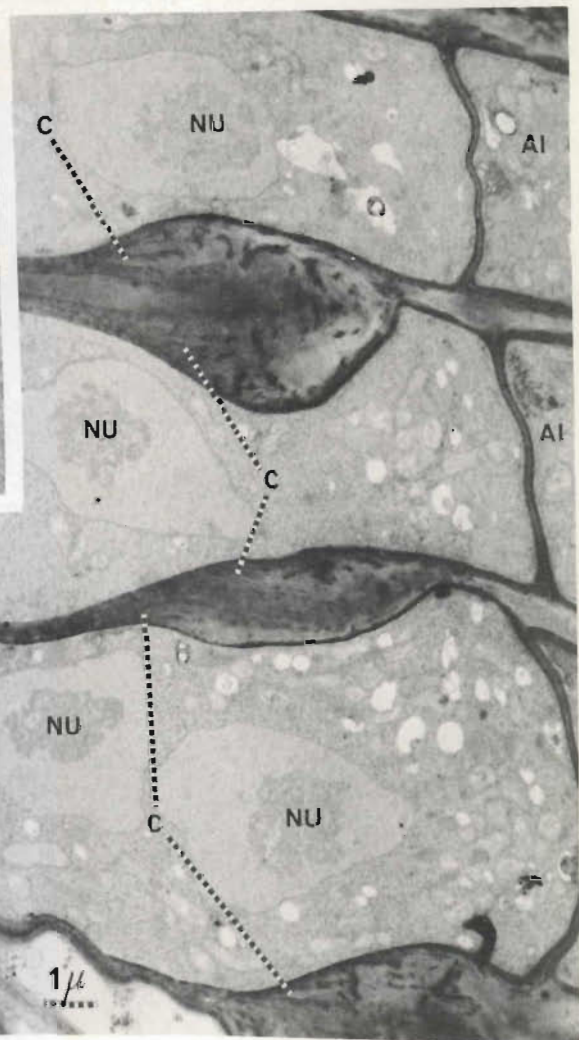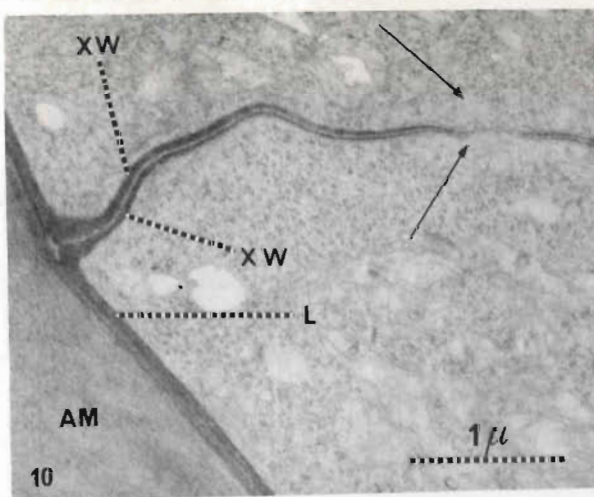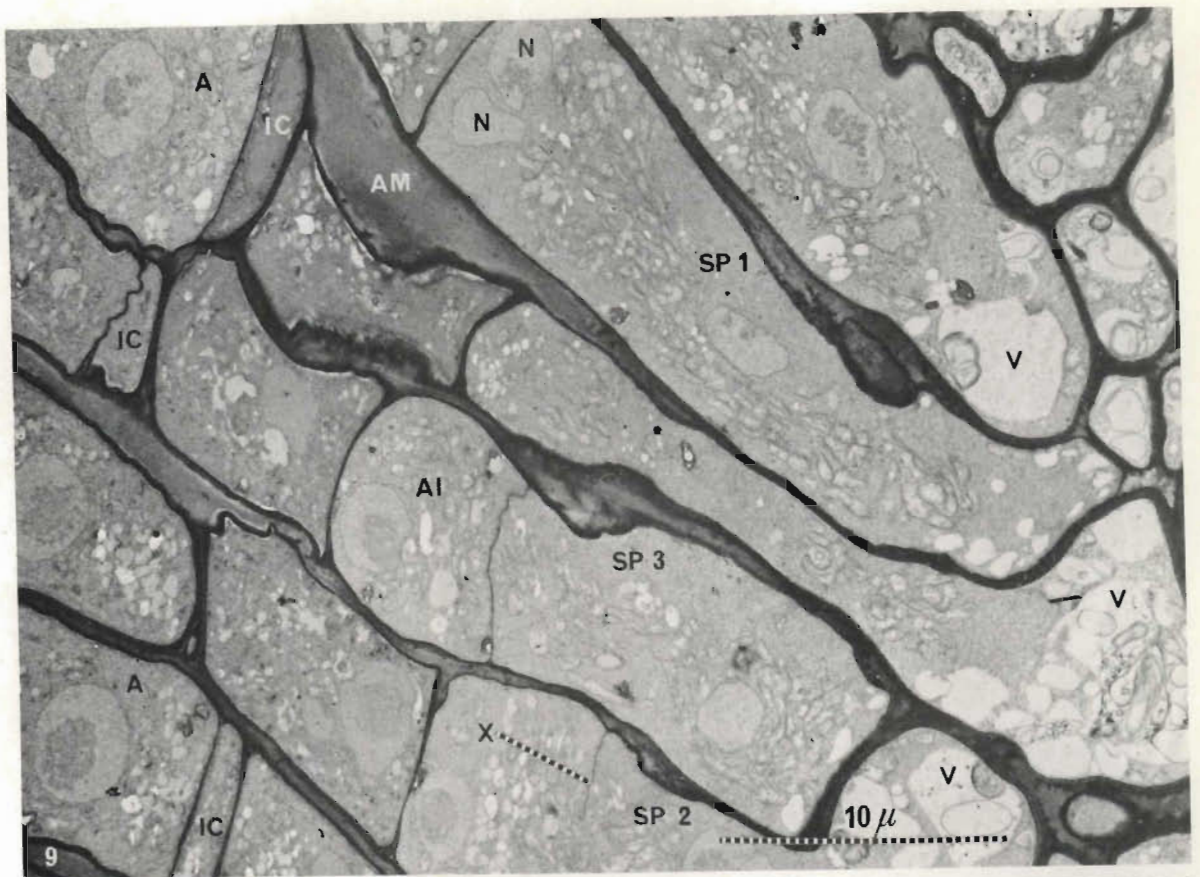
Figs. 9–11. 9) Aeciosporophores showing developmental stages in production of aeciospore initials (AI). After division daughter nuclei (N) have migrated to apical part of sporophore (SP1). Centripetal cross-wall (X) formation (SP2) results in delimitation of aeciospore initial from sporophore (SP3). Wall of sporophore base is thicker than wall of distal part. Note intercalary cells (IC), aeciospores (A) and aecial matrix (AM). 10) Septum of sporophore - aeciospore initial, showing electron-lucent septal lamella separating electron-opaque septal walls (XW) that widen considerably at junction with periclinal wall.
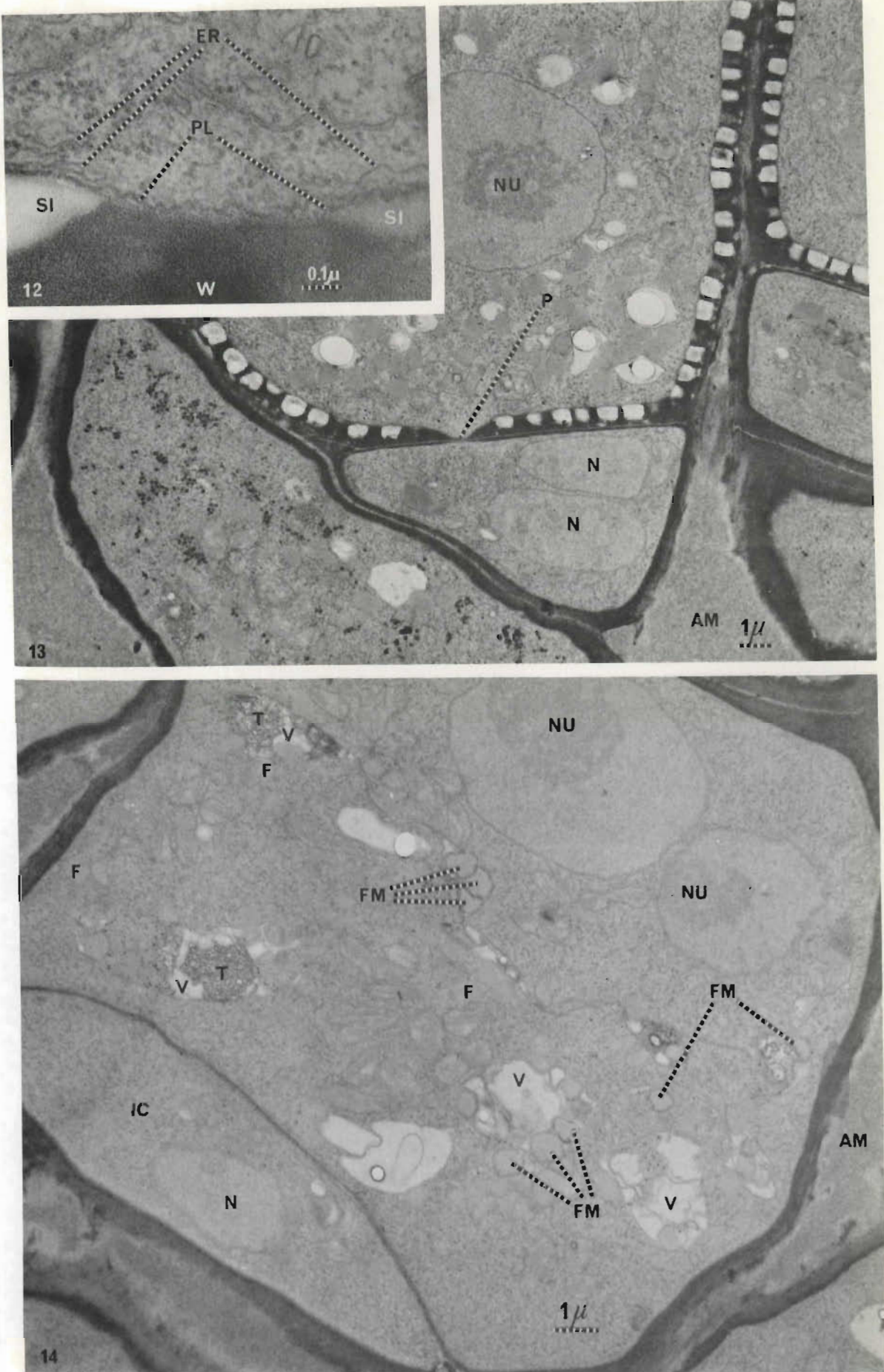
Figs. 12-14. 12) Early stage of spine development in aeciospore. Spine initials (SI) form between plasmalemma (PL) and cell wall (W). ER lamellae lie in close proximity to initials. 13) Tangentially sectioned pore (P) in aeciospore - intercalary cell septum. Note binucleate (N) condition of intercalary cell, and, in aeciospore wall, funnel-shaped channel leading to pore. 14) Section of immature aeciospore showing vesicle-containing structures (T) closely associated with vacuoles (V), and membrane-enclosed (FM) and non-enclosed (F) fat globules. Note intercalary cell (IC) and holes in section. AM = aecial matrix; N = nucleus; NU = nucleolus.
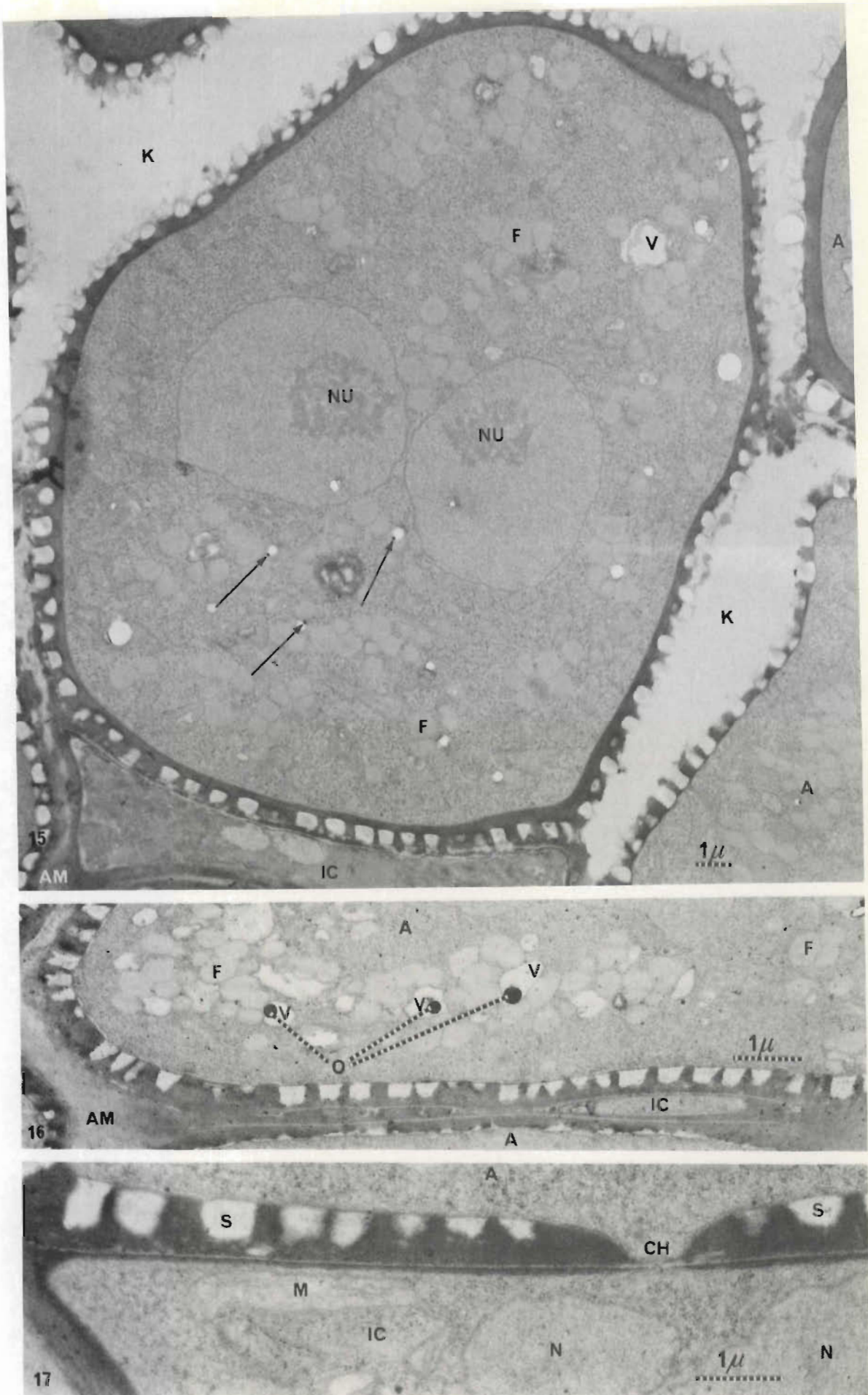
Figs. 15-17. 15) Young aeciospore after emergence from aecial matrix (AM) into aecial cavity (K). Spines have become separated from plasmalemma by wall deposition. Nucleoli (NU) at this stage are still clearly resolvable. Note holes that appear to have formed at electron-dense granules (arrows) in small vacuoles. 16) Part of young aeciospore showing vacuoles (V) containing electron-opaque granules (O). 17) Off-centre section of septum between young aeciospore and intercalary cell. Channel (CH), leading to pore, in wall of aeciospore is filled with cytoplasm. Note normal appearance of cytoplasm and cytoplasmic components of
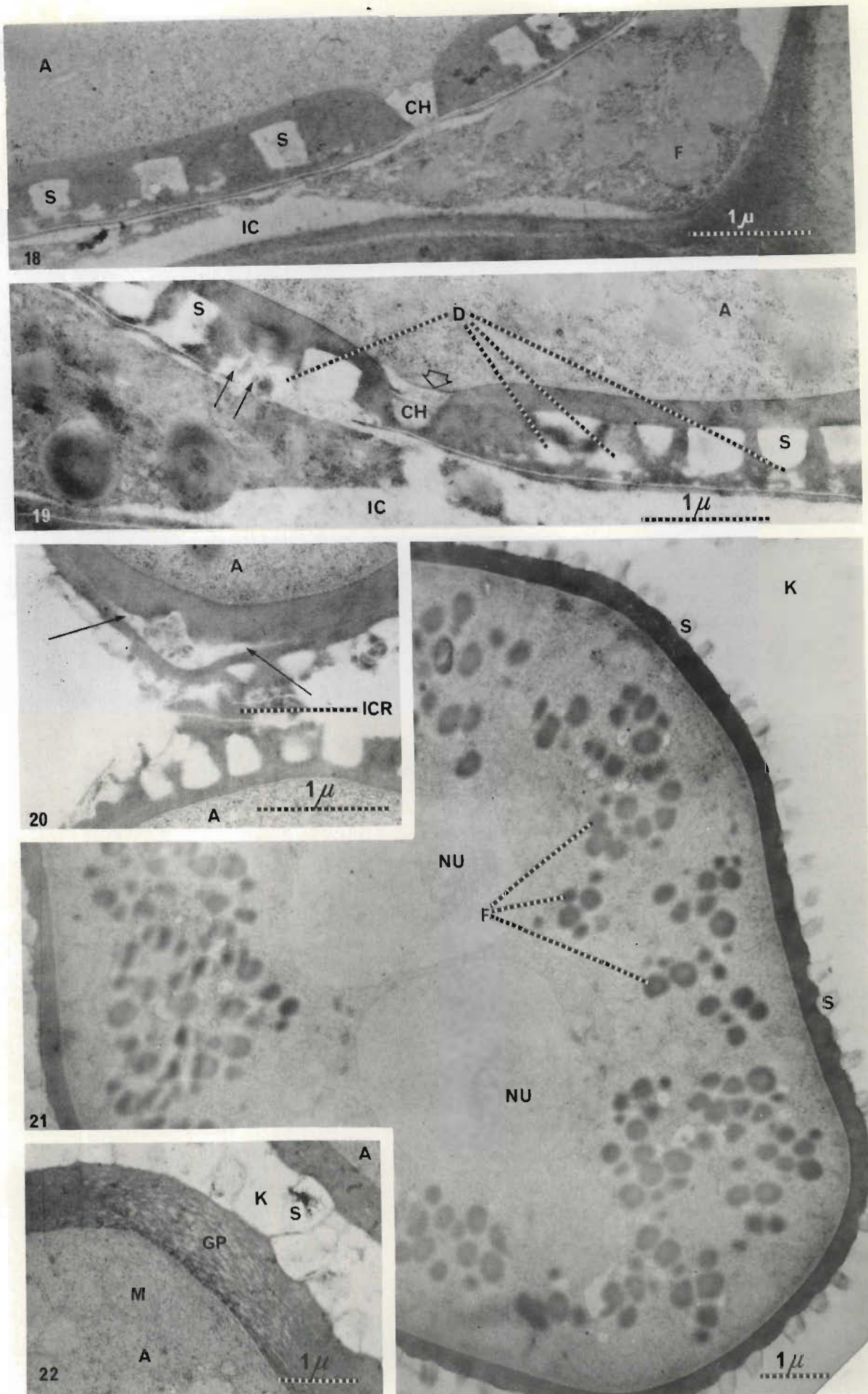
Figs. 18-22. 18) Retraction of cytoplasm from aeciospore channel (CH) at stage when cytoplasm of intercalary cell (IC) becomes degenerate. 19) Early stage in sealing off of aeciospore channel (CH) by wall apposition (open arrow). Note (i) advanced cytoplasmic degradation and incipient wall disintegration of intercalary cell (IC), (ii) disintegration (D) of wall material between, and covering, aeciospore spines, and (iii) granules (arrows) associated with wall disintegration. 20) Parts of two mature aeciospores showing that wall layers fail to fuse (arrows) in vicinity of channel. Note traces (ICR) of intercalary cell residue and indentation

three-layered (Fig. 2). The outer layer is substantial and confluent with outer layers of contiguous cells. Many of the basal cells are uninucleate and vacuolate (Fig. 3). Similar cells extend around the aecium (Fig. 4), grading into extensively vacuolate prosenchymatous cells that are separated from the peridium by a residue of compressed cell debris (Fig. 5).

The peridium is composed of a single layer of binucleate cells, the walls of which are markedly thickened where they adjoin the compressed residue (Fig. 5). In these thickened parts of the wall are embedded electron-transparent processes that are bizarrely shaped with forked and more or less curved, tapering tips (Figs. 5, 7). On the peridial walls bordering the aecial cavity, spine-like projections extend from the plasmalemma through the wall and protrude into the cavity (Figs. 6, 7). Nuclei in peridial cells are almost spherical (Figs. 6, 7) and nucleolate (Fig. 7). The cytoplasm includes glycogen, ribosomes, many fat globules and short mitochondria (Fig. 6).

Amongst the uninucleate cells in the aecial base, and in the zone between the base and sporophores, large irregularly shaped multinucleate cells, that contain electron-dense inclusions, are found (Fig. 8).

The wall of a mature sporophore is thicker in the proximal than in the distal region (Fig. 9). After division of the two prominently nucleolate sporophore nuclei, the daughter nuclei migrate to the distal part of the sporophore (Fig. 9 - SP 1) and a crosswall delimiting the aeciospore initial (Fig. 9 - SP 3) is laid down centripetally between the two sets of nuclei (Fig. 9 - SP 2). A central pore, in which a pulley-wheel apparatus has in some instances been observed, is retained (Fig. 10). The cytoplasm sur-

rounding pores is devoid of organelles (Fig. 10). Where the electron-dense septal walls, separated by an electron-transparent lamella, join the periclinal wall they widen considerably. The lower septal wall is continuous with the inner layer of the sporophore wall (Fig. 10). This layer extends to form a subsequent aeciospore initial in the basipetal chain and ruptures the periclinal wall which then flares outwards. Detachment of successive aeciospore initials gives rise to a series of wall remnants, or annellations, that are borne at approximately the same level on a mature sporophore (Fig. 11).

After formation of a septum that cuts off the aeciospore initial, nuclear division takes place in the preceding initial and a septum is laid down delimiting a young binucleate aeciospore from a small wedge-shaped binucleate intercalary cell (developmental stages are shown in Fig. 1). We could not establish whether the pore of this septum has a pulley-wheel apparatus. Soon after septum formation development of electron-lucent spines is initiated between the plasmalemma and the wall of the immature aeciospore. ER lamellae lie in the spore cytoplasm in close proximity to the developing spines (Fig. 12), which remain enveloped by progressive apposition of wall material. Eventually the spines become separated from the plasmalemma by a wall layer (Fig. 15) which increases in thickness even after spore release into the aecial cavity. The septal wall tapers to the pore forming a funnel-shaped channel (Figs. 13, 18).

While the aeciosporophore base is markedly vacuolate (Fig. 9), distally the sporophore contains small vacuoles and some fat globules (Fig. 11). The small vacuoles increase in size in the aeciospore initial and in the young aeciospore until spine development has been completed. The enlarging

vacuoles and vesicle-containing structures are often associated with membrane-enclosed or non-enclosed fat globules (Fig. 14) that increase greatly in number during this stage of spore development. Electron-dense granules are common in vacuoles during this developmental stage (Fig. 16). Almost invariably holes appear in sections of developing aeciospores (e.g. Figs. 13, 14, 15) and seem to originate at the site of electron-dense granules (Fig. 15).

Aeciosporophores, aeciospore initials, intercalary cells and immature spores are embedded in a matrix which, in electron-density, resembles the aforementioned outer layer of cell walls in the aecial base. Degradation of intercalary cells (Fig. 18) and of the inter- and supraspinal wall of aeciospores (Fig. 19) commences prior to spore release from the matrix into the aecial cavity. Small, well-defined granules are found in parts of the wall that are undergoing disintegration (Fig. 19 - arrows). The cytoplasm of the funnel-shaped channel in the aeciospore wall (Fig. 17) retracts during disintegration of the intercalary cell (Fig. 18). Cell wall deposition continues (Fig. 19) and in the process the channel is sealed off by a thick wall layer that fails to fuse with the material previously apposed around the channel (Fig. 20). At the centre of this area, opposite the channel, the thick inner layer is consistently indented (Fig. 20).

Nucleoli, still easily recognizable in spores approaching release (Fig. 15), fade in electron-density upon spore secession and are progressively difficult to demonstrate as aeciospores mature (Fig. 21). Aeciospore cytoplasm contains many fat globules, ER, ribosomes and short mitochondria. Germ pores, in which the normal wall matrix is interspersed with electron-lucent

material, are formed in the spore wall (Fig. 22).

Inter- and intracellular hyphae of the aecial stage are often very vacuolate and frequently contain degenerate cytoplasm.

Discussion

The wall of cells in the base of the aecial stroma was found to possess a thick outer layer which resembles that of pseudoparenchymatous cells of Uromyces appendiculatus uredia (Müller, Rijkenberg & Truter, 1974 a) and Puccinia sorghi pycnia (Chapter 2). As mentioned in the previous chapter such thick wall layers may play a role in the water balance of rust fructifications. Walls of peridial cells, markedly thickened where they adjoin compressed cell residue, may contribute to such a role in aecia. The cell debris presumably originates from compression of fungal and host cells by the post-fertilization development of the aecium.

The uninucleate nature of (i) many cells in the base of the stroma, and (ii) prosenchymatous cells around the layer of cell debris, indicates that these cells are derived from pre-fertilization monokaryotic mycelium. Allen (1934) has averred that, during fertilization, pycniospore nuclei of P. sorghi "multiply and spread rapidly" from the point of entrance into the haploid thallus, and found, presumably from a random survey, that diploidization had been achieved in 60% of all thallus cells 24 hr after pycniospore application. We found no evidence of such diploidization in vegetative hyphae; the binucleate condition, with few exceptions, appears to be restricted to cells found in the aecium.

Savile (1939) reported that "staining reactions show that much of the ergastic material in cells that have performed their function or that have

only a mechanical function, such as the peridial cells, is removed for use in the more active cells". In the present study it was found that cells which have performed their function are extensively vacuolate, but the cytoplasm of peridial cells is densely filled with organelles and fat globules. Continued metabolism of peridial cells ensures the manufacture of the thick wall that characterizes this cell type. The electron-lucent processes embedded in thickened parts of peridial cell walls appear as striations when entire cells are viewed under a light microscope. The manner in which these processes originate is similar to that of the spine-like projections on the part of the walls facing the aecial cavity.

In the present study multinucleate cells were very evident. This type of cell will be discussed in detail in Chapter 4.

In essence the findings concerning aeciospore development reported in the present study agree with the excellent light microscopic description of this process in the same fungus by Savile (1939). It is difficult, however, to correlate this author's observations regarding the nuclear "endo-" and "ectosphere" with the electron micrographs shown in the present study. No comparisons of mitotic figures are possible for, although hundreds of sporophores were scanned, the present author could find no division figures of nuclei. The interphase nuclei of sporophores, clearly shown in Fig. 11, are of the "expanded" type described by Savile. Such "expanded" nuclei measure approximately 5 - 6μ in diameter and possess proportionally large nucleoli, measuring approximately 3μ in diameter. If "ectosphere membrane" be equated with nuclear membrane and "endosphere" with nucleolus, a membrane surrounding the nucleolus, as suggested by Savile, was not

observed in the present investigation. "Unexpanded" nuclei, in sections of which small nucleoli are often observed, are found in monokaryotic, intercalary and peridial cells and measure $3 - 4\mu$ in diameter.

Spore ontogeny is unusual in that, in addition to aeciospores, intercalary cells are formed from aeciospore initials that arise as percurrent prolifera-tions. Remnants of periclinal walls, formed by the secession of successive aeciospore initials, are borne at approximately the same level on the sporo-phore and constitute a collar around mature aeciosporophores. These rem-nants, or annellations, are comparable to those of P. sorghi pycniosporophores described in Chapter 2.

In a description of aeciospore ontogeny based on several light micro-scopic studies, Hughes (1970), as most other authors, referred to the aecio-sporophore as a "basal cell" from which, by successive elongation and division, a basipetal succession of aeciospore initials is formed. On this ba-sis he classified aeciospores as meristem arthrospores, belonging to Section V (Hughes, 1953) of the conidiophore/conidium groupings. On account of its well-developed collar of annellations the present author considers develop-ment of aeciospore initials to be annellophoric, and proposes that the desig-nation "aeciosporophore", rather than "basal cell" be used.

In light microscopic studies of P. sorghi (Allen, 1934; Rice, 1933; Savile, 1939) no mention is made of the matrix that envelops sporophores, aeciospore initials, intercalary cells and young spores. The present author also could not resolve this component in a preliminary light microscopic investigation of P. sorghi aecia (unpublished observations) and ascribes this to a lack of resolving power rather than to the fixation or wax-embedding

techniques employed. No function can be assigned to this substance, but it may be similar to the "intercellular material" in the aecial stroma of P. podophylli (Moore, 1963 a) and strongly resembles the "thick primary wall" of P. poarum described and shown by Henderson, Prentice & Eudall (1972). The terminal phase of intercalory cell disintegration coincides with its emergence from the aecial matrix and releases the spore into the aecial cavity. From electron micrographs of Uromyces caladii aecia published by Moore & McAlear (1961) it is obvious that cells they labelled "disjunctor cells" retain their cellular integrity in the aecial cavity.

It is interesting that the occurrence of enlarging granule-containing vacuoles, and closely associated vesicle-containing structures, coincides with a considerable numerical increase of membrane-enclosed and non-enclosed fat globules in their immediate vicinity. While it is not possible at this stage to propose a mechanism for the formation of fat globules, the associated cytoplasmic components appear to mediate, or at least be implicated in, the increase in their number. It is noteworthy that there is a close structural similarity between vesicle-containing structures of this stage and those that contain tubules in the pycnial stage (Chapter 2).

Wall ornamentation of aeciospores at the ultrastructural level has been well documented and illustrated by a number of authors (e.g. Grand & Moore, 1972; Henderson, Prentice & Eudall, 1972; Hiratsuka, 1971; Moore & McAlear, 1961; Von Hofsten & Holm, 1968; Walkinshaw, Hyde & Van Zandt, 1967). The development of aeciospore spines in P. sorghi is similar to that of uredospore spines in Melampsora lini (Littlefield & Bracker, 1971) in as far as (i) spine initials are first observed as electron-

lucent areas between plasmalemma and wall, and (ii) ER lamellae are close-
ly associated with developing spines. Henderson, Prentice & Eudall (1972)
have described and illustrated the association of ER with developing spines
of P. poarum aeciospores. Unlike the spines of M. lini (Littlefield &
Bracker, 1971) and U. appendiculatus (Müller et al., 1974 a) uredospores,
spines of P. poarum (Henderson, Prentice & Eudall, 1972) and P. sorghi
aeciospores do not push through the wall. The latter authors refer to inter-
spinal aeciospore wall and aecial matrix collectively as the "primary wall",
which, in their opinion, is removed, probably by reabsorption into the spore.
In P. sorghi only wall material between, and covering, spines is removed.
In these inter- and supraspinal parts of the wall, granules occur that are
similar to those associated with the development of teliospore ornamentation
(Henderson, Eudall & Prentice, 1972) and teliospore germ pores (Henderson
et al., 1972; Müller et al., 1974 b) and appear to be involved in enzy-
matic wall degradation.

Aeciospore spines become separated from the plasmalemma by wall appo-
sition, whereas in peridial cells such apposition does not occur. The chan-
nel that leads to the septal pore of the spore base is partially plugged by
an electron-dense substance in uredospores of Uromyces appendiculatus (Mül-
ler et al., 1974 a) but the funnel-shaped channel in P. sorghi aeciospores
is sealed off by cell wall deposition. Germ pores of P. sorghi aeciospores
are not as prominent as those shown in micrographs of some other rusts (Von
Hofsten & Holm, 1968). Spore cytoplasm, and the organelles it contains,
is similar to that found in other types of rust spores. No reason can be
advanced for the fading in electron-density of nucleoli in mature aeciospores.

No dictyosome-resembling membrane complexes of the type occurring in P. podophylli (Moore, 1963 b) were observed in P. sorghi.

The vacuolate and often degenerate nature of cells that constitute the inter- and intracellular hyphae, and the outer zone of the P. sorghi aecium, probably indicates that accumulated food reserves in the aecium require little or no supplementation during sporulation. To investigate this aspect further it might be interesting to assess the extent and duration of sporulation by young aecia after their dissection from host tissue. On the other hand, the possibility cannot be discounted that, as has been suggested in the case of intercellular hyphae (Chapter 1), the aecium utilises host diffusates.

CHAPTER 4

C E L L   F U S I O N   I N   T H E   A E C I U M   O F
P U C C I N I A   S O R G H I

Introduction

Allen (1934), Rice (1933) and Savile (1939) have observed multinucleate cells in the base of aecial primordia and/or aecia of <u>Puccinia sorghi</u> Schw. Allen (1934) stated that: "Rarely, and under conditions little understood, a haploid aecium attains greater size and development before degeneration sets in. In these aecia occur monstrous teratological, multinucleate cells of most irregular form and indeterminate growth. These cells soon die and the aecium as a whole degenerates". She made no reference to the occurrence of such cells in the spore-producing aecium, nor to cell fusion in P. sorghi. According to Rice (1933), who studied the same species, "Binucleate cells are seen early in the very base of the aecidium while among them and above them multinucleate cells also appear early in the development of the anlage. I did not find evidences of Christman fusions. There are many fusions and occasional migrating nuclei between irregular lobes of the crowded cells". Savile (1939) could find no evidence of septal dissolution in any material of the <u>Oxalis</u> stage of this fungus and did not observe cells that contained more than four nuclei. In Chapter 3 it has been reported that, at the ultrastructural level, multinucleate cells in aecia of this species were very evident.

In the present chapter a more detailed description of the multinucleate,

or fusion, cell type and its relationship to aeciosporophores is given.

Materials and Methods

Material embedded to study the aecial stage was used to examine cell fusion, and procedures similar to those described in Chapter 3 were followed.

Observations

In a cross-section of the aecial base (Fig. 1) four main cell types can be clearly differentiated: (i) uninucleate, often vacuolate cells that are common in the base of the stroma (Fig. 1 - B), (ii) binucleate sporophores, with thick walls (c.f. Chapter 3) and electron-lucent cytoplasm, sectioned longitudinally (Fig. 1 - SP 1), obliquely (Fig. 1 - SP 2) and occasionally through a proximal extension, or foot (Fig. 1 - SP 3), (iii) multinucleate fusion cells, recognisable by their often irregular shape, nuclear condition, dense cytoplasm and electron-opaque cytoplasmic inclusions (Fig. 1 - F), and (iv) cells with degenerate cytoplasm and large vacuoles containing a granular residue (Fig. 1 - G).

In many multinucleate cells remnants of septa are discernible.   The structure of septa and infolded walls, or pseudosepta, in P. sorghi is in all respects similar to that of Uromyces appendiculatus described by Müller, Rijkenberg & Truter (1974).   The structural differences between septal remnants and infolded walls are schematically shown in Fig. 2.
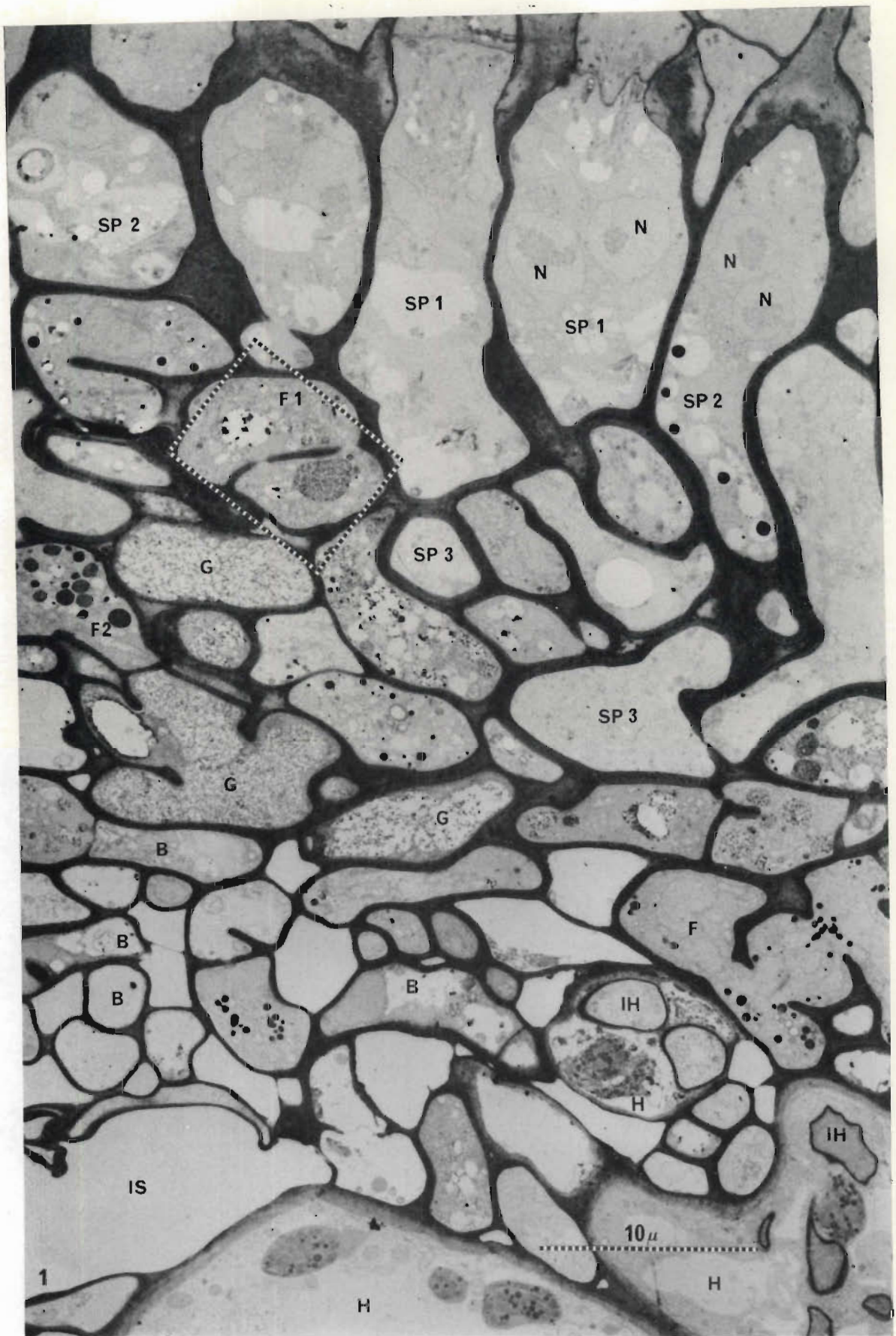
Fig. 1. Section through aecial stroma of Puccinia sorghi. Four cell types can be clearly differentiated: (i) monokaryotic vacuolate cells (B) in base of aecial stroma, (ii) binucleate (N) sporophores (SP1), several of which (SP2) have been sectioned obliquely or sectioned through their basal extensions (SP3), (iii) fusion cells (F) and (iv) cells (G) with degenerate cytoplasm and residue-containing vacuoles. IH = intracellular hypha; IS = intercellular space; H = host cell.
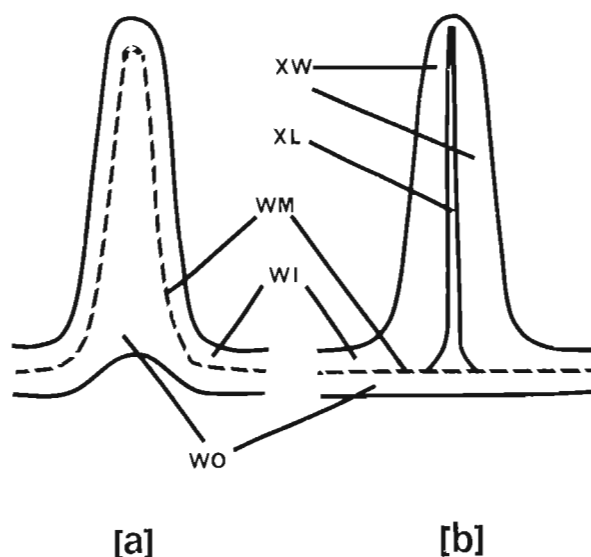
Fig. 2. Schematic comparison between (a) infolded wall and (b) septum or septal remnant. Note how, in infolded wall, middle layer (WM) follows contour of inner layer (WI), and outer layer (WO) coalesces in fold. At septum. or septal remnant, middle (WM) and outer (WO) wall layer are not infolded, and septal walls (XW), separated by septal lamella (XL), are continuous with inner layer (WI).

Usually not all identifying characteristics can be clearly distinguished, but in most instances, compare for example septal remnant in Fig. 3 with infolded wall in Fig. 11, differentiation is not difficult. Septum degeneration does not necessarily commence at the pore, but is often initiated eccentrically (Fig. 3 - double-headed arrow). Partial breakdown of septa allows the migration of nuclei from cell to cell (Fig. 4).

While the exact number of cells that partake in the formation of a fusion cell could not be ascertained, it is evident from serial sectioning that at least three consecutive cells may be involved. Figs. 5, 6 and 7 are serial sections showing apparently entire septa (Fig. 5) which, in another plane (Figs. 6, 7 - arrows), are partially degraded. Partial septa are occasionally irregular in shape and/or manner of attachment to a periclinal wall (Fig. 7). Up to seven nuclei per cell have been seen in sections of fusion cells.
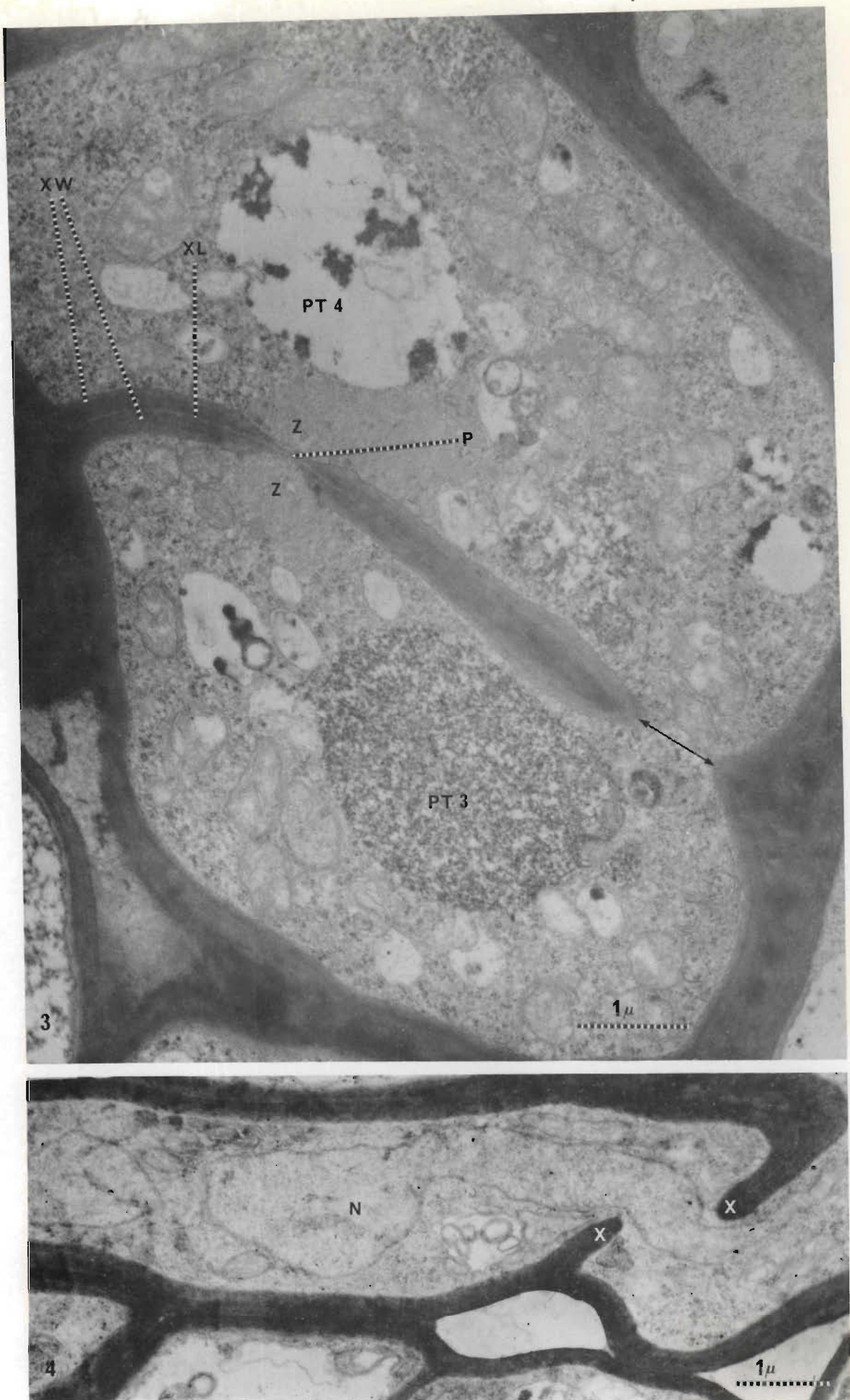
Figs. 3-4. 3) Section of septum in fusion cell (F1) boxed in Fig. 1. Pore (P) is enveloped by hemispherical zone (Z) of organelle-free cytoplasm. During fusion, aperture has formed in septum against periclinal wall (double-headed arrow). Note septal walls (XW) and lamella (XL), and proteinaceous structures (PT 3 and 4). 4) Migration of nucleus (N) between cells after partial dissolution of septum (X).
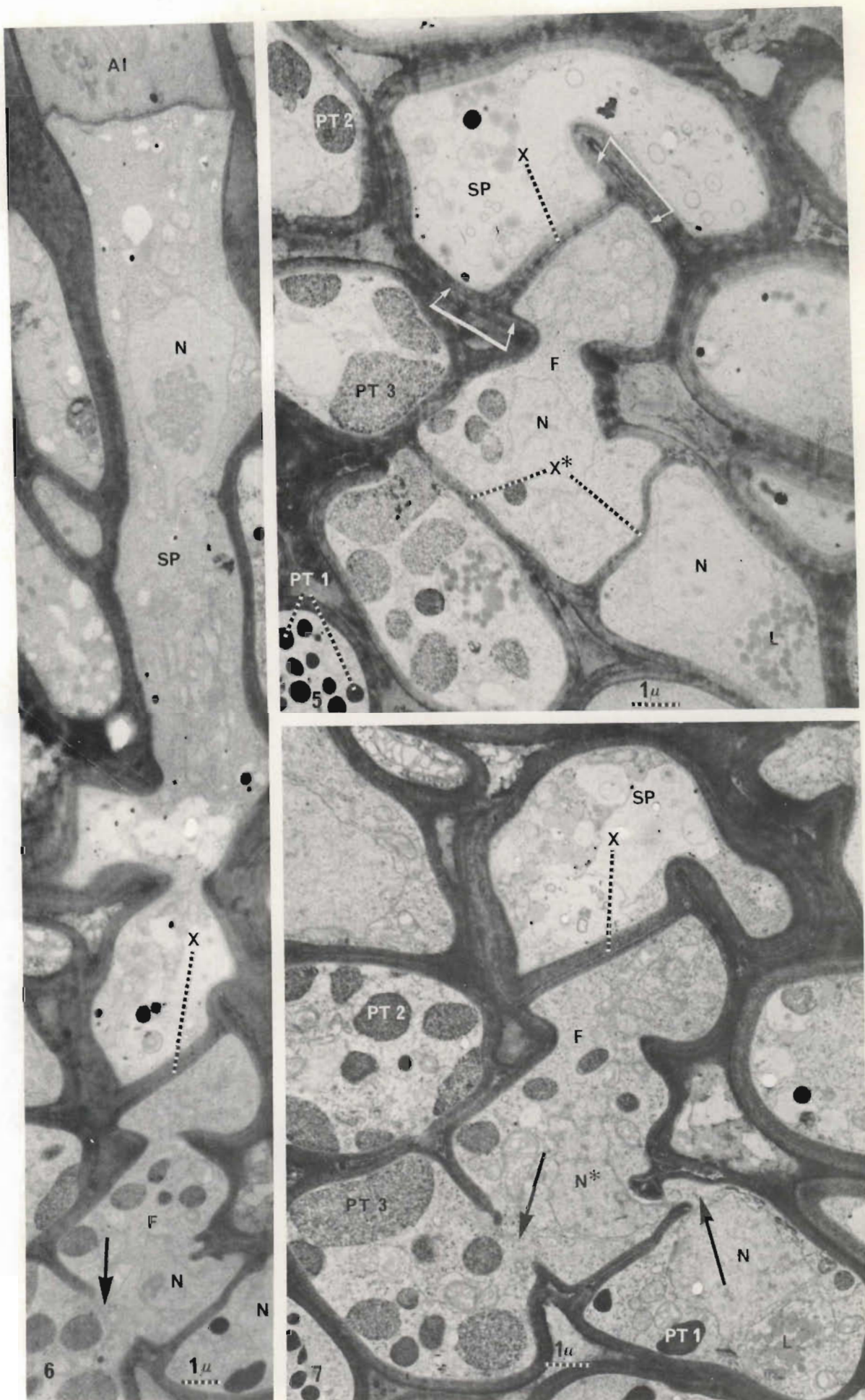
Figs. 5, 6 (montage) and 7. Serial sections of fusion cell (F) separated by septum (X) from sporophore (SP). Continuity of periclinal wall (between white arrows) at sporophore-fusion cell septum in Fig. 5 indicates sporophore is derived from fusion cell. Septa (X*) shown as entire in Fig. 5 are partially degraded in Figs. 6 and 7, and have large apertures (arrows). In Fig. 7 septal remnants in fusion cell are irregular in shape and manner of attachment. Note proteinaceous structures (PT 1 - 3), nuclei (N), one of which (N*) in Fig. 7 appears to be migrating through septal aperture, and SP
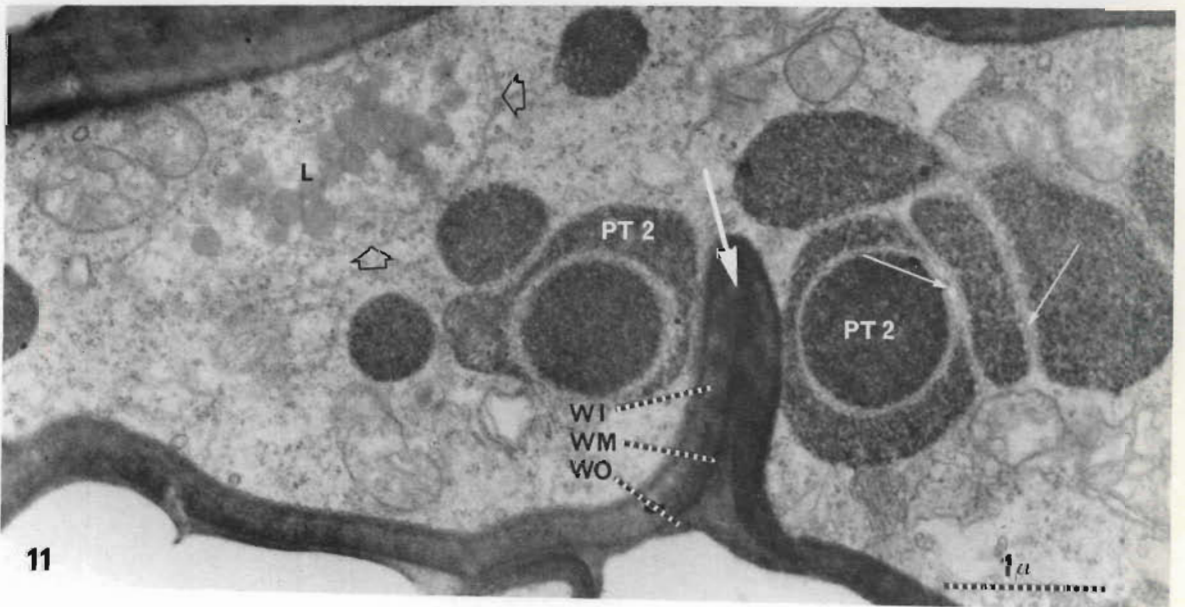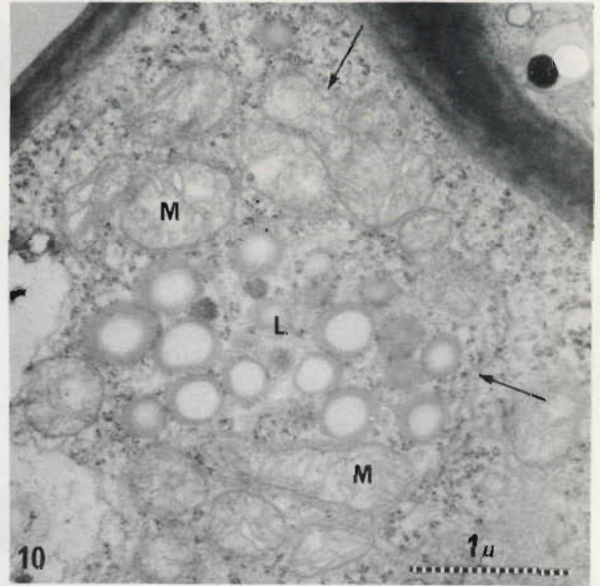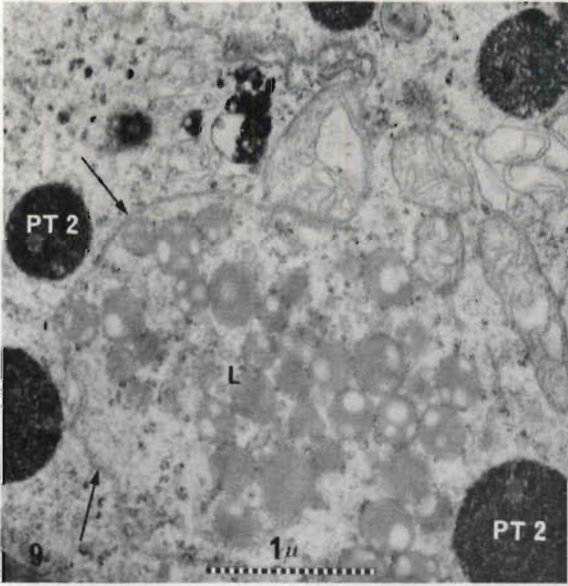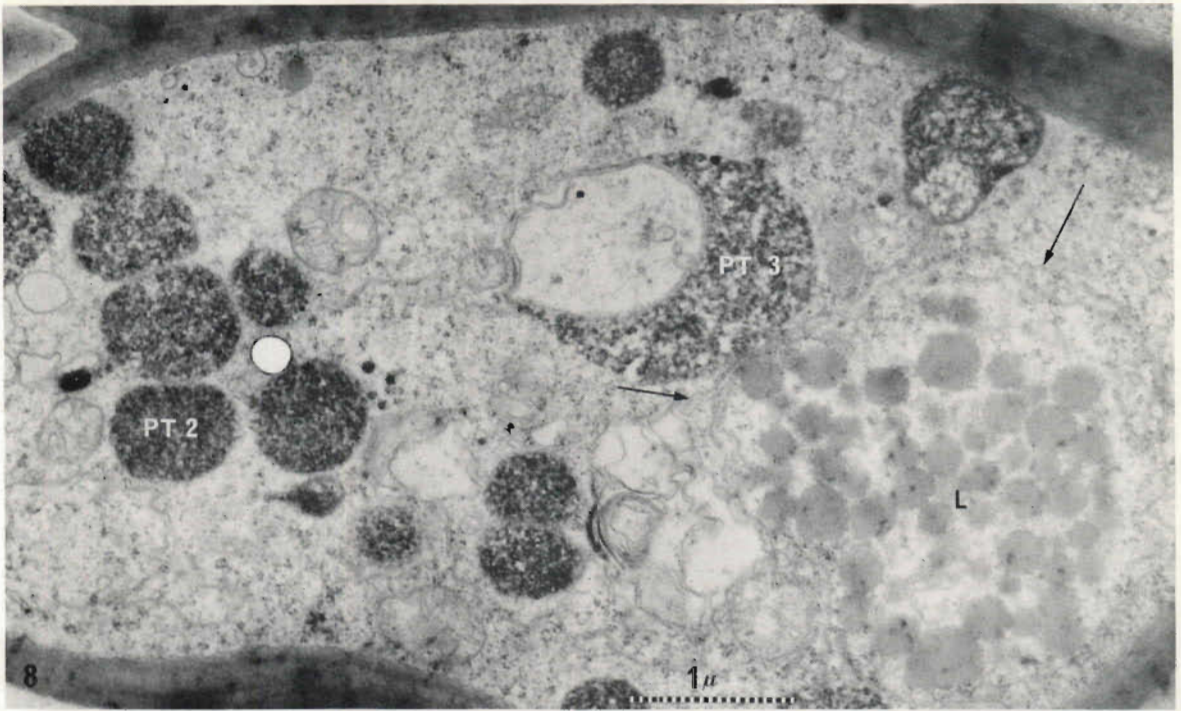
Figs. 8-11. 8) Part of fusion cell showing electron-opaque proteinaceous structures (PT2 and 3) and ER-enclosed (arrows) aggregate (L) of lipid bodies. 9) Part of fusion cell showing electron-opaque proteinaceous structures (PT 2) and ER-enclosed (arrows) aggregate (L) of lipid bodies, of which several possess one or more electron-transparent lacunae. 10) Part of fusion cell showing ER-enveloped (arrows) aggregate (L) of lipid bodies each with large central lacuna. Note mitochondria (M) in close association with lipid bodies. 11) Part of fusion cell showing ER-enveloped (arrow
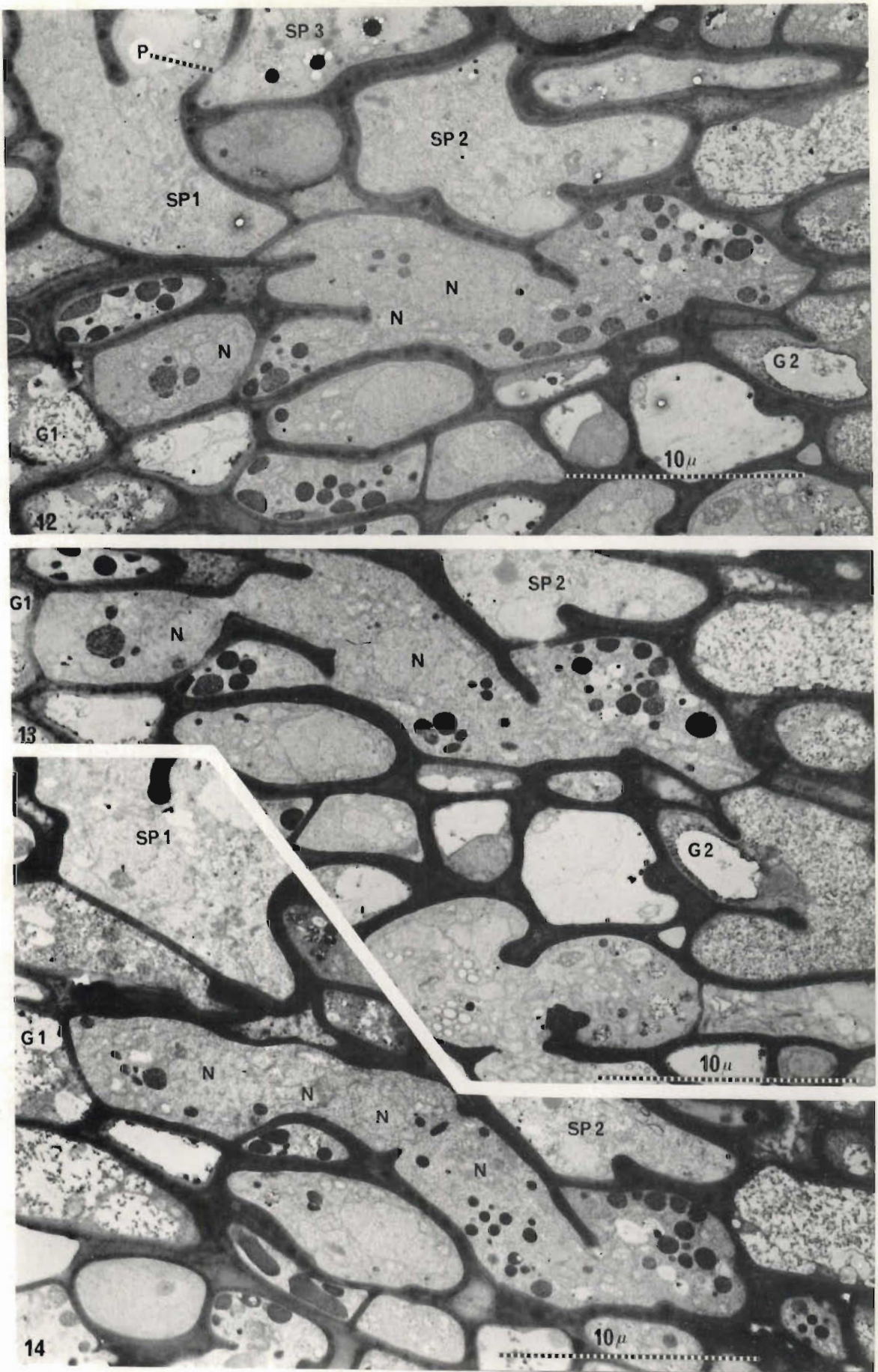
Figs. 12-14. Serial sections of fusion cell (F2) of which part is shown in Fig. 1. Multinucleate (N) cell is separated by septa from two cells (G 1 and 2) with degenerate cytoplasm, and two sporophores (SP1 and 2) Note pore (P) connecting SP1 to SP3 in Fig. 12.
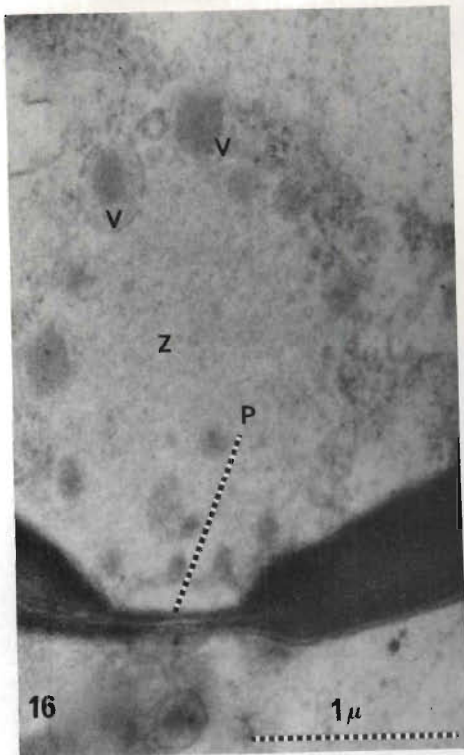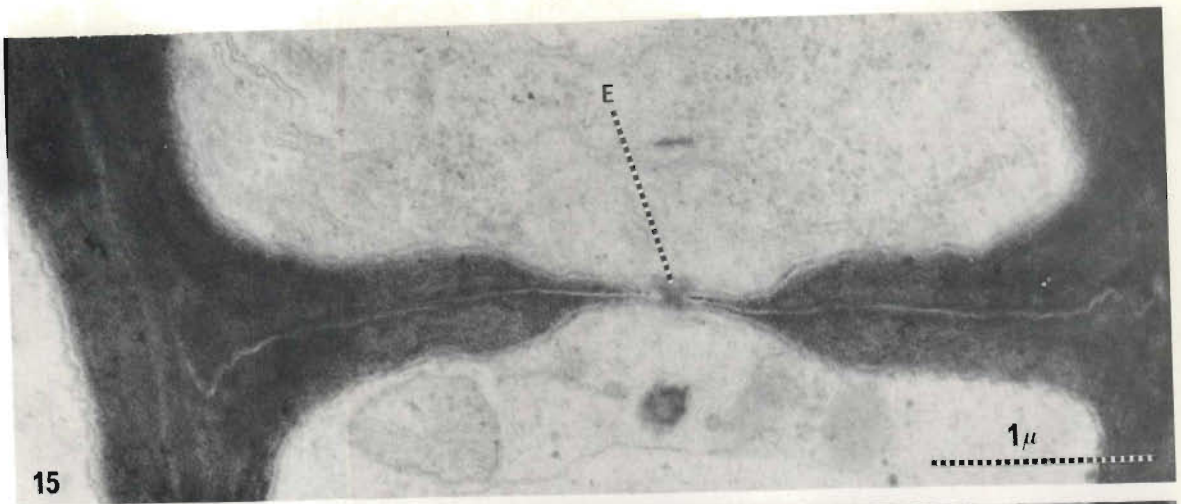
Figs. 15-17. 15) Section of septal pore in base of aecial stroma plugged with electron-dense material (E).
16) Zone of organelle-free cytoplasm (Z), with associated crystal-containing vesicles (V) at periphery, after
withdrawal from pore (P) which is tangentially sectioned. 17) Multinucleate (N) fusion cell (F) with typical
cytoplasmic components (L and PT 4). Note sporophore - sporophore septal pore (open arrow). Pore (black
arrow), between fusion cell and vacuolate sporophore extension, has been covered by wall layer. Inset of

The cytoplasm of fusion cells contains two characteristic types of components. One type of inclusion is, initially, an aggregate of typical lipid bodies surrounded by lamellae of endoplasmic reticulum (Figs. 8, 11). Each lipid body in the ER-enveloped aggregate develops electron-lucent lacunae (Figs. 9, 17) which progressively coalesce to form one central lacuna (Fig. 10). In some instances mitochondria, together with lipid bodies, are also enclosed by lamellae of endoplasmic reticulum (Fig. 10). These aggregates were also occasionally seen in monokaryotic hyphae in the immediate vicinity of the aecium. A second type of cell component, which is sometimes clearly membrane-bounded (Fig. 11), shows the following variations: (i) an electron-opaque angular to near-spherical structure (Figs. 5, 7, 9 - PT 1), (ii) less electron-opaque forms (Figs. 5, 7, 8, 11 - PT 2), (iii) large rounded bodies with granular contents (Figs. 3, 5, 8, 9 - PT 3), and (iv) vacuole-like structures containing loose electron-dense aggregates (Figs. 3, 17 - PT 4). These structures may enfold others (Fig. 11).

A multinucleate cell in Fig. 1 (F2) is shown at a higher magnification in Figs. 12, 13 and 14 which are micrographs of serial sections. Separated from this cell by a septum is the aforementioned cell type (G) with degenerate cytoplasm and large vacuoles containing granular residue. Also separated from the fusion cell by a septum are two cells (SP 1 and 2) which, on the basis of the characteristics of the wall and cytoplasm, are sporophore extensions (Fig. 12). Separation of a sporophore from a fusion cell by a septum is also evident in Figs. 5, 6 and 7. Sporophore extension SP 1 in Fig. 12 is, in turn, connected by a pore to another sporophore extension, SP 3. Such sporophore - sporophore pores were often seen (see also

Fig. 17). In the aecial stroma, pores may become plugged by an electron-dense substance (Fig. 15), and the, in rusts commonly occurring, hemispherical zone of organelle-free cytoplasm with crystal-containing vesicles may withdraw from the pore area and become near-spherical (Fig. 16). In some instances a plugged pore between a vacuolate sporophore base and a multinucleate cell is sealed off in the latter cell type by a layer of wall material (Fig. 17 and inset).

Discussion

The present investigation furnishes evidence of cell fusion in the aecial stroma of Puccinia sorghi and shows that fusion may be followed by nuclear migration.

Blackman (1904) first described the role of nuclear migration in the dikaryotisation of aecia. In a comprehensive cytological study of Phragmidium violaceum (Schultz) Wint. he found the aecial primordium immediately beneath the leaf epidermis to consist of a layer of cells, each cell forming a distal sterile cell, the proximal or "fertile" cell becoming "fertilised" by migration into it of the nucleus of an undifferentiated mycelial cell at its base. The mycelial and "fertile" cell were either consecutive cells in a hypha or contiguous cells of different hyphae. Although he did observe the migration of the nucleus as a narrow thread during its passage from one cell to another, the aperture, due to its small size, could not be resolved. Normally two, rarely three, on one occasion four, nuclei were observed in a "fertile" cell, which, soon after nuclear migration, commenced a series of rapid divisions, forming aeciospore initials.

A different type of dikaryotisation was described by Christman (1905) who examined the aecial stages of three other rust species. In an early developmental stage he observed division of vertically orientated cells into a sterile cell and a larger basal cell. Unlike Blackman (1904) he frequently saw, after such division, the formation of a "pore" between two adjoining basal cells and the union of the upper halves of protoplasts by gradual enlargement of the pore, the binucleate structure, after conjugate nuclear division forming aeciospore initials.

Both Blackman (1904) and Christman (1905) considered the processes they described to be sexual fusions in homothallic organisms. A flurry of papers, in which workers reported similar fusions in other rusts, appeared during the subsequent two or three decades and it seemed that the vexing problem of sexuality in the Uredinales had been solved. Olive (1908) showed how the seemingly divergent accounts of Blackman and Christman could be reconciled and contributed the following important consideration: "The apparently normal and regular occurrence at the base of certain young aecidia of one to many multinucleated cells, points to the necessity of a broader conception as to the mode of development of the aecidium-cup than that held by either Blackman or Christman. While the part which these multinucleated cells take in the development of the aecidium is as yet somewhat obscure, the evidence appears to point to the conclusion that they are sporophytic structures and that they result from the stimulated growth which follows the sexual cell fusions".

Multinucleate cells in the aecial base have been reported frequently [see Allen (1930) and Rice (1933) for discussions on this topic]. In an early

developmental stage of _Puccinia sorghi_ aecia, Rice (1933) found "many fusions and occasional migrating nuclei between the irregular lobes of the crowded cells", but could find no evidence of the regular type of fusions described by Blackman and Christman. Whereas Rice (1933) reported the occurrence of multinucleate cells in the binucleate stage, Allen (1934) found no evidence of fusion in the same species and saw multinucleate cells in the monokaryotic stage only, while Savile (1939) observed neither septal dissolution nor cells containing more than four nuclei. Allen (1930) in an investigation on _P. graminis_ and Rice (1933) in her study on _P. sorghi_ presented evidence that the "basal cell" of the dikaryotic aecial stage arises from a multinucleate cell.

Allen (1934) has shown that multinucleate cells of _P. sorghi_ originate in the monokaryotic aecial primordium and the multinucleate condition, therefore, is not dependent on the introduction of a pycniospore nucleus into the thallus. The present author found that multinucleate cells in young aecia may arise from fusion of at least three, and probably more, sequential cells, and up to seven nuclei per cell have been seen in sections of these cells. Whereas Allen (1934) reported that multinucleate cells, seen by her in aecial primordia only, soon died, the present author considers that the state of the cytoplasm indicates that considerable metabolic activity characterises such cells in aecia.

Andrus (1933), in a study on _Uromyces_ spp., stated that the degree of dissolution of cell walls during cell fusion in aecial primordia is almost complete, and that in the dikaryotised aecium only localised dissolution, or rupture, of cell walls is evident when cells fuse. The present author

found that septal degeneration in fusion cells of young P. sorghi aecia is, generally, in an advanced stage. From observations in the present investigation it is evident that septal degeneration is an important mechanism in the formation of fusion cells, but the possibility that infolded walls, prevalent in the stroma and in multinucleate cells, are remnants of contiguous cell walls, may not be excluded. The irregular structure, and the manner of attachment to periclinal walls, of some septal remnants cannot be explained. Cells (Fig. 13 - G), attached to multinucleate cells and characterised by degenerate cytoplasm and residue-containing vacuoles, may be cells of the original hypha that were not incorporated in the fusion cell, or "sterile" cells similar to those referred to by Blackman (1904) and Christman (1905).

From her observations Rice (1933) inferred that aeciospore chains of P. sorghi originate from lobes of multinucleate cells. The present study shows that sporophores are initially produced by fusion cells, and the presence of sporophore - sporophore septa and pores indicates that such primary sporophores, by basal branching, form secondary sporophores. Since it is the fusion cell that gives rise to primary sporophores, and as it is generally accepted that sporophore nuclei are genetically different and complementary, it seems obvious that the pycniospore nucleus finds its way from the point of entrance on a flexuous hypha to this cell in a manner still requiring elucidation, but entailing little, if any, dikaryotisation of the mycelium through which it passes (c.f. Chapter 3). Previously, Craigie (1959) and Craigie & Green (1962), in studies on P. helianthi and P. graminis respectively, had indicated that division of the introduced pycniospore nucleus during its passage probably occurs rarely. As a multinucleate cell gives rise

to more than one sporophore, it is probable that the pycniospore nucleus undergoes division in this cell type. Whether multinucleate cells in an aecial primordium require introduction into the thallus of one or several pycniospore nuclei is not known, but it is interesting to note that Olive (1908) observed nuclear migration between multinucleate cells of P. cnici. Cytoplasmic continuity between a fusion cell and an associated sporophore may be severed by the deposition of a wall layer over the pore site.

Although ER-enveloped aggregates, composed of lipid bodies possessing electron-transparent lacunae, were occasionally seen in monokaryotic hyphae in the immediate vicinity of the aecium, they are more frequently found to be cytoplasmic components of multinucleate cells. It would appear that, during their active metabolism, these cells mobilise the core of the lipid body as an energy source, while a shell of lipid is retained. The electron-opaque proteinaceous structures, observed in fusion cells only, in appearance resemble lysosomes found in Ceratocystis fimbriata (Wilson, Stiers & Smith, 1970), but are also similar to structures such as secretory granules in rabbit parotid (Castle, Jamieson & Palade, 1972) and many other types of glandular cells, as well as yolk platelets in eggs (e.g. Schroeder, 1972). Whether these cytoplasmic components serve in the storage of protein, or whether they are true lysosomes and play an enzymatic role, for example in the breakdown of septa, remains to be established. These cytoplasmic inclusions have, as far as is known, not previously been observed in ultrastructural investigations of rusts.

Except for some noteworthy exceptions in the 1930 - 1940 period, research on cell fusions and multinucleate cells in aecial stages has been largely

neglected since Craigie (1927) demonstrated the function of pycnia and pyc-niospores. Furthermore, it is surprising that, almost half a century after Craigie's findings, the manner in which the pycniospore nucleus reaches the aecial primordium and initiates dikaryotisation still remains obscure. The present study sheds no light on this aspect, but does indicate that cell fusion plays an important role in the terminal phase of the dikaryoti-sation process.

GENERAL SUMMARY

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 at least 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 haustorium-like 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".

Sporogenesis in pycnia of P. sorghi is annellophoric, but at secession the spore retains the septal lamella and at least part of the lower septal wall. Pycniospores are bounded by a thin ($\pm$ 85 nm) wall and contain a nucleolate nucleus, endoplasmic reticulum, tubule-containing structures, several short mitochondria with predominantly plate-like cristae, many ribosomes and a few lipid bodies. Septal pores were sectioned infrequently in pycnia, but more commonly in inter- and intracellular hyphae. All were equipped with a pulley-wheel apparatus.

In the aecium of P. sorghi the substantial outer layer of cell walls in the base of the stroma, together with the thick wall of peridial cells where they adjoin the compressed cell debris, may play a role in the water balance of the fruiting structure. With few exceptions binucleate cells are found only in the aecium. Cytoplasm of the binucleate peridial cells is densely filled with organelles and fat globules. Large multinucleate cells with inclusions, that are apparently proteinaceous in nature, are found in the aecial base. The ontogeny of aeciospore initials is annellophoric and aeciosporophores have a well developed collar consisting of annellations. Sporophores, aeciospore initials, intercalary cells and young aeciospores are enveloped by a matrix. During aeciospore ontogeny fat globules are formed in the immediate vicinity of enlarging vacuoles and vesicle-containing structures, suggesting that the formation of fat globules is associated with these cytoplasmic components. There is a close structural similarity between the vesicle-containing structures of this stage and those that contain tubules in the pycnial stage. Spine initials are first observed as electron-lucent areas between the plasmalemma and the wall of immature aeciospores. During

further development the inter- and supraspinal wall of the spore is degraded, a process in which small, well-defined granules appear to be implicated. The spines become separated from the plasmalemma by wall apposition. Apposition also seals off the channel leading to the septal pore in the aeciospore base. The spore wall contains several germ pores. The vacuolate and often degenerate nature of inter- and intracellular hyphae in thalli with young sporulating aecia points to accumulation of food reserves in young aecia.

Ultrastructural evidence of cell fusion, and associated nuclear migration, in the aecial stroma of P. sorghi is presented and the observations are discussed in relation to light microscopic investigations conducted by other authors. Fusion is effected by degeneration of septa and at least three consecutive cells may be involved. The multinucleate fusion cell is, presumably, the site of division of the pycniospore nucleus before the cell forms binucleate sporophores. Cytoplasmic continuity between a fusion cell and an associated sporophore may be severed by the deposition of a wall layer over the pore site. Proteinaceous structures and ER-enveloped aggregates, the latter composed of lipid bodies that typically possess one or more lacunae each, are common cytoplasmic components of multinucleate cells.

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