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ELECTRON MICROSCOPIC OBSERVATIONS ON HAUSTORIA ISOLATED FROM CUCUMBER LEAVES INFECTED WITH POWDERY MILDEW[1]

Elektronenmicroscopische waarnemingen aan haustoriën, geïsoleerd uit met echte meeldauw geïnfecteerde komkommerbladeren

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The fine structure of haustoria isolated from cucumber leaves infected with powdery mildew (*Sphaerotheca fuliginea*) was studied. Electron microscopy showed a haustorial body of 5–7 μ which contains many mitochondria and is surrounded by an electron lucent sheath of up to 4 μ in thickness. The sheath is limited by a heavily invaginated membrane of about 0.03 μ. The central body is connected with the sheath membrane by fingerlike convoluting protrusions.

INTRODUCTION

Recently DEKHUIJZEN (1966a) described a method for the isolation of haustoria from cucumber leaves infected with powdery mildew (*Sphaerotheca fuliginea*). The isolated haustoria were able to consume oxygen and to reduce the stain Janus green B.

Microscopic examination showed granules in the dense central body and in the more diffuse outer layer (sheath) of the haustoria. According to HIRATA (1937) and HIRATA & KOJIMA (1962), protrusions are formed at the ellipsoidal central body of *S. fuliginea*. These authors point to the fact that these tangled and fingerlike protrusions in the sheath are often misinterpreted as granules during microscopic examinations since they grow convolutely. For this reason the electron microscope has been used in this study to investigate more closely the internal structure of the central body and the sheath.

Metabolic studies of the isolated haustoria are valuable only when the haustoria are free from host cytoplasm. For this reason particular attention has been paid to the outher sheath membrane, since host particles might be attached to this membrane. A short communication of this study has already been given earlier by DEKHUIJZEN (1966b).

MATERIALS AND METHODS

About 60×10^6 haustoria were isolated from cucumber leaves according to a method described in a previous paper (DEKHUIJZEN, 1966a). They were fixed in 6% glutaraldehyde in 0.1 M Sorensen phosphate buffer at pH 6.8 for two

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hours and washed three times with the buffer by centrifugation. The pellet was mixed with 2% agar. The coagulated agar was cut into small blocks and steeped overnight in 1% osmium tetroxide in the same buffer. After washing in the buffer and dehydrating with ethanol, the blocks were embedded in Vestopal W via styrene (KURTZ, 1961). However, the mixture of styrene and Vestopal (1:1) was not brought under vacuum and the final embedding was polymerized during 48 hours at 60° C.

Sections, about 600–900 Å thick, were cut with a Porter-Blum ultramicrotome, using a glass knife. The sections were stained with lead citrate according to REYNOLDS (1963), sometimes preceded by staining in a saturated solution of uranyl acetate in 50% ethanol. Occasionally a 2% potassium permanganate staining was used.

The electron micrographs were taken with a Siemens Elmiskop 1.

RESULTS

A general view of the haustorium

Fig. 1 shows a general view of a section of an isolated haustorium. The center consists of an ellipsoidal haustorial body with a diameter of 5–7 μ. The body has lobes (Figs. 1, 2) and is limited by a relatively thick wall. The wall is surrounded by an electron lucent sheath which varies from 2 to 4 μ.

The sheath is limited by a sheath membrane. The holes in the sheath membrane may be due to damage during the isolation procedure.

The islands in the sheath are sections of protrusions of the haustorial body. Evidence for this opinion will be given below.

The haustorial body and its protrusions

The haustorial body contains a large number of mitochondria (Figs. 1, 2). Their cross-sections vary from 0.5 to 1 μ, and they are characterized by numerous cristae. Electron dense droplets, which may contain lipids, frequently occur.

The nature of the vesicles (Fig. 2, 4) is not clear, but they may be secretion vesicles produced by the Golgi apparatus. However, a Golgi apparatus has not been found. BRACKER (1964a,b) also did not observe a Golgi apparatus in haustoria of *Erysiphe graminis*. The nucleus has not yet been observed clearly in the electron micrographs of the isolated haustoria.

The cytoplasm of the body is limited by a plasma membrane of approximately 75 Å (Fig. 2). In one of the micrographs (Fig. 5) the membrane appears to be three-layered. A three-layered plasma membrane has not yet been described for haustoria of *E. graminis* (EHRLICH & EHRLICH, 1963a) or *Puccinia graminis* (SHAW & MANOCHA, 1965) but it is known to occur in plants (GRUN, 1963).

The plasma membrane is surrounded by a relatively thick wall of about 0.1 μ which consists of a fine granular or fibrillar structure (Figs. 2, 5).

In most cases the haustorial body wall is separated from the sheath membrane by the sheath. Fig. 4, however, shows a part of a body wall which is in close contact with the sheath membrane.

The electron dense particles between the sheath membrane and haustorial wall and the one at the inside of the wall may suggest a movement of this material from the fungus to the host or the reverse. Movement of vesicles has been suggested from electron micrographs by EHRLICH & EHRLICH (1963b) and

PEYTON & BOWEN (1963). However, care must be taken in making such suggestions from static micrographs.

The islands in the sheath are limited by a membrane and wall similar to that of the haustorial body (Fig. 5). Both the haustorial body and the islands contain mitochondria (Fig. 3) and droplets (Fig. 5). Therefore it must be concluded that the islands are sections of protrusions of the haustorial body. Since these protrusions run very convolutely from the central body to the sheath membrane they are only rarely completely visible in one section. In most cases they are visible as lobes of the haustorial body or as islands in the sheath.

Often the protrusions can be seen at short distances from the sheath membrane (Figs. 1, 6), whereas Fig. 3 shows a close contact of the wall of a protrusion with the sheath membrane. The sheath membrane appears to be invaginated at the place of contact with the protrusion wall as well as on places where no contact is visible.

The neck of the haustorium

The neck of the haustorium is that part of the pathogen which connects the haustorial body with the external hypha (BRACKER, 1964b). Fig. 6 shows part of this neck.

The neck of the haustorium contains a septum which is also visible in the electron micrographs of the haustorium of *E. graminis* published by EHRLICH & EHRLICH (1963a) and in Fig. 7. According to BRACKER (1964a,b), however, the septum of the haustorium of *E. graminis* contains a central pore that may be plugged at both sides or open. A central pore is not visible in Figs. 6 and 7, but the granular electron dense material near the septum may be identical to that of the plugged material described by BRACKER. The internal structure of the cytoplasm in Fig. 6 is not well preserved. The isolated haustoria differ in age and it might be possible that this is a relatively old haustorium in which the pore has been plugged before the haustorium lost its function.

The sheath

The sheath consists of an electron lucent substance whose structure does not differ very much from that of the embedding medium (Figs. 1, 3, 5), but sometimes small aggregations of fine granular material are visible in the amorphous sheath (Fig. 5). The thickness of the sheath is never greater than 4 μ.

According to EHRLICH & EHRLICH (1963a) the sheath of *E. graminis* consists of an electron lucent background substance in which electron opaque material is embedded, whereas BRACKER (1964a,b) observed a fine granular material in the amorphous sheath. HIRATA & KOJIMA (1962) observed that the volume of the sheath could be varied by addition of lithium. The absence of clear cytoplasmic structures and the fact that there is no clear collapse of the sheath when part of the sheath membrane is damaged during the isolation procedure (Fig. 1) may suggest that the sheath consists of a highly viscous substance.

The sheath membrane

The sheath membrane is often visible as a heavily invaginated membrane which runs more or less parallel to the central body (Figs. 1, 5) and can be folded back over large distances into the body of the sheath (Fig. 6). Fig. 8, however, shows that protrusions of the sheath membrane also occur.

The formation of protrusions and invaginations may have important functions in the uptake of nutrients from the host.

DISCUSSION

This electron microscopic study confirms the view of HIRATA (1937) and HIRATA & KOJIMA (1962) that fingerlike protrusions are formed at the ellipsoidal body of *S. fuliginea*. In the case of *E. graminis* the protrusions extend straight over relatively large distances, whereas the protrusions of *S. fuliginea* grow very convolutely.

According to the Japanese workers the protrusions of *S. fuliginea* are only formed at the basal and distal end of the haustorial body, and they should grow convolutely along the surface of the haustorial body. However, Fig. 3 does not lend support to this opinion, since it shows a close contact between the wall of the protrusion and the sheath membrane. Although Fig. 1 gives no definite proof, it must be doubted whether the protrusions can only be formed at the distal and basal end of the haustorial body. It might be possible that the protrusion can be formed from any point of the body.

Recently, MCKEEN *et al.* (1966) made an electron microscopic study of *Erysiphe cichoracearum* haustoria in sunflower. These haustoria appeared also to contain an ellipsoidal body with protrusions. However, the authors believe that the protrusions emerge only from the distal and basal end of the haustorial body.

The sheath membrane is heavily invaginated. This has also been observed on the sheath membrane of *E. graminis* (EHRLICH & EHRLICH, 1963a; BRACKER, 1964a,b). HIRATA & KOJIMA (1962) believe that this membrane is of host origin. EHRLICH & EHRLICH (1963a), however, give very strong evidence for the view that the sheath membrane of *E. graminis* is at least partly of fungal origin since their micrographs show a sheath membrane which runs parallel to the penetration peg and extends through the host wall. This opinion is not shared by BRACKER (1964b). He considers the sheath membrane as an extension of the host ectoplast and the sheath as the zone between haustorial wall and host ectoplast.

Definite proof for the origin of the sheath membrane cannot be given from this study of the isolated haustoria, but it is surprising that this membrane appears to be firmly attached to the sheath, and in most cases the membrane is completely free from the host plasma membrane and host cytoplasm. The isolation of the haustorial body intact with the sheath and sheath membrane indicates that not only the body but also the sheath and the sheath membrane are probably of fungal origin.

In most cases the sheath membrane appeared not to be seriously damaged during the isolation procedure. Therefore it must possess unusual toughness. Further evidence of the stability of the sheath membrane comes from the work of HIRARA & KOJIMA (1962) who were able to pull haustoria from host cells. The sheath remained intact and retained its properties of differential permeability.

Many questions concerning the fine structure of the haustoria remain unsolved, but this study shows that the internal structure of the haustoria is not seriously damaged by the isolation procedure. This result is in agreement with the fact that the isolated haustoria consume oxygen and reduce Janus green (DEKHUIJZEN, 1966a), which is probably due to the activity of the mitochondria.

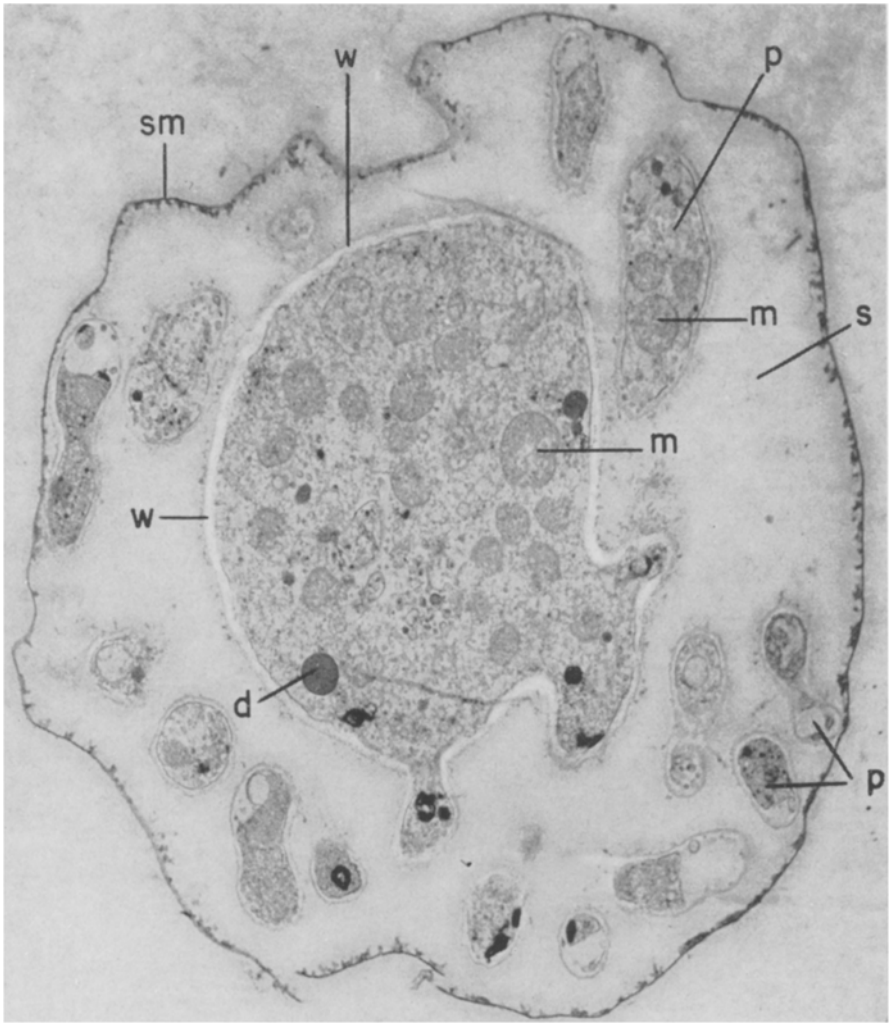

FIG. 1. A section of an isolated haustorium of *Sphaerotheca fuliginea* stained with lead citrate. Magnification × 10,000.

Een doorsnede van een geïsoleerd haustorium van Spaerotheca fuliginea, *gekleurd met loodcitraat. Vergroting 10.000 ×.*

Explanation in the figures:
Abbreviations: d=droplet; e=electron dense particle; h=haustorial body; hp=haustorial plasma membrane; i=invagination; m=mitochondrion; n=neck of haustorium; p=protrusion of haustorial body; pl=plug; s=sheath; se=septum; sm=sheath membrane; v=vesicle; w=wall of the haustorial body; wp=wall of protrusion of haustorial body.

Verklaringen in de figuren:
Afkortingen: d=druppel; e=elektronen verstrooiend materiaal; h=centrale lichaam; hp=membraan van het haustoriumcytoplasma; i=instulping; m=mitochondrium; n=hals van het haustorium; p=uitstulping van het centrale lichaam; pl=prop; s=schede; se=septum; sm=schedemembraan; v=blaasje; w=wand van het centrale lichaam; wp=wand van de uitstulping van het centrale lichaam.

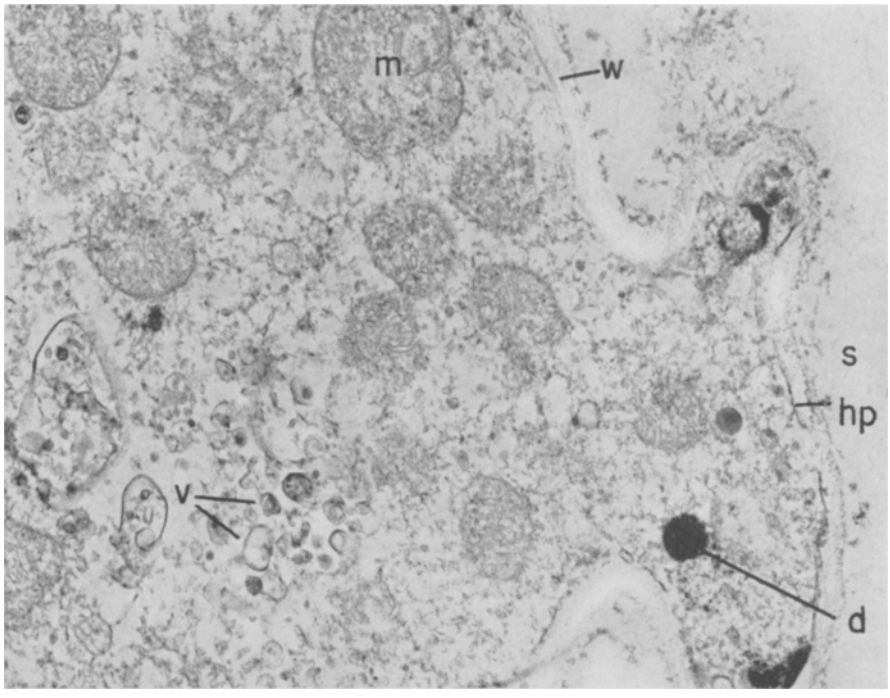

FIG. 2. Part of haustorial body. Detail of Fig. 1. Magnification × 23,000.
Een gedeelte van het centrale lichaam. Detail van fig. 1. Vergroting 23.000 ×.

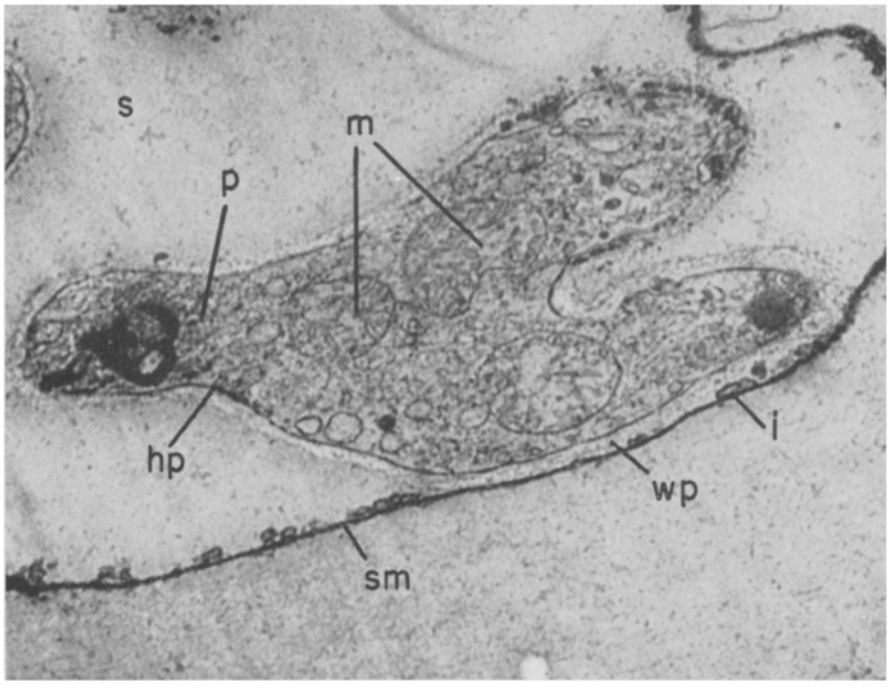

FIG. 3. Contact between wall of protrusion of haustorial body and the invaginated haustorial sheath membrane. Stained with $KMnO_4$. Magnification × 40,000.
De wand van een uitstulping van het centrale lichaam in aanraking met de geïnvagineerde schedemembraan. Kleuring $KMnO_4$. Vergroting 40.000 ×.

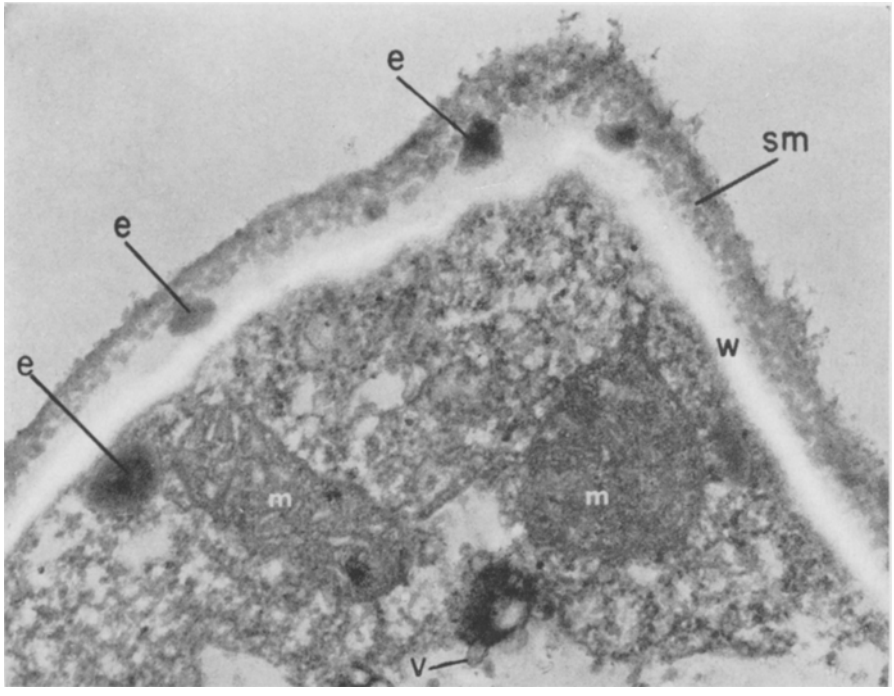

FIG. 4. Contact between the haustorial body wall and the sheath membrane. Stained with lead citrate and uranyl acetate. Magnification × 60,000.

De wand van het centrale lichaam in aanraking met de schedemembraan. Kleuring met loodcitraat en uranylacetaat. Vergroting 60.000 ×.

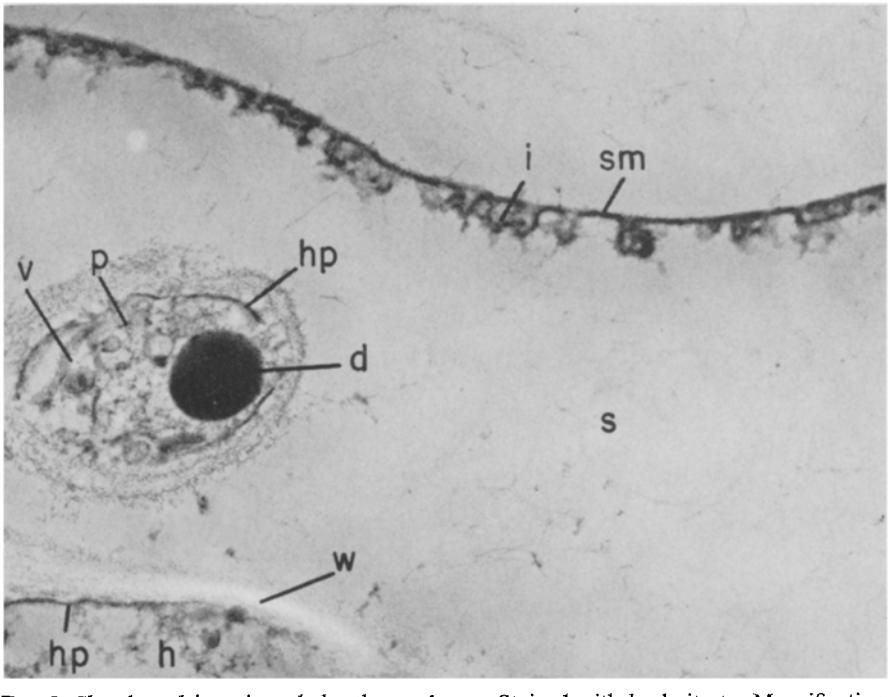

FIG. 5. Sheath and invaginated sheath membrane. Stained with lead citrate. Magnification × 40,000.

Schede en geïnvagineerde schedemembraan. Kleuring met loodcitraat. Vergroting 40.000 ×.

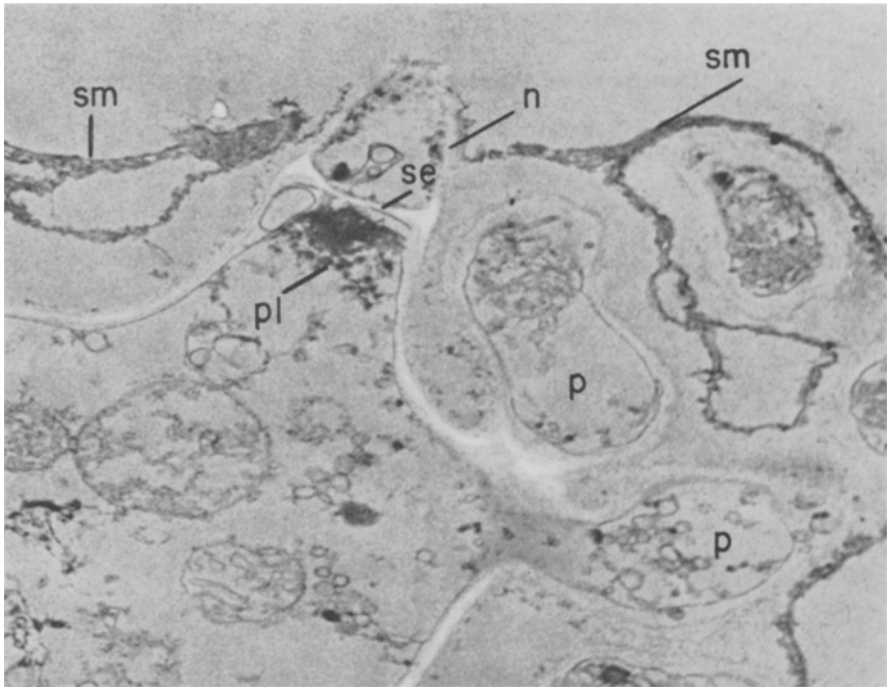

FIG. 6. Neck of haustorium with septum. Stained with lead citrate. Magnification × 22,000.
Hals van het haustorium met septum. Kleuring met loodcitraat. Vergroting 22.000 ×.

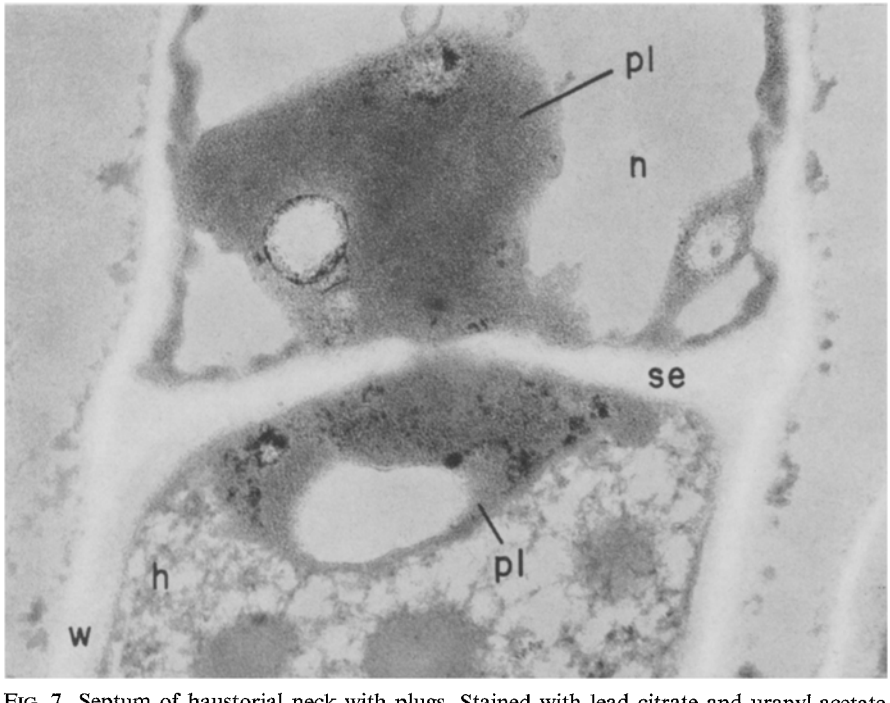

FIG. 7. Septum of haustorial neck with plugs. Stained with lead citrate and uranyl acetate. Magnification × 80,000.
Septum van de hals van het haustorium met proppen. Kleuring met loodcitraat en uranylacetaat. Vergroting 80.000 ×.

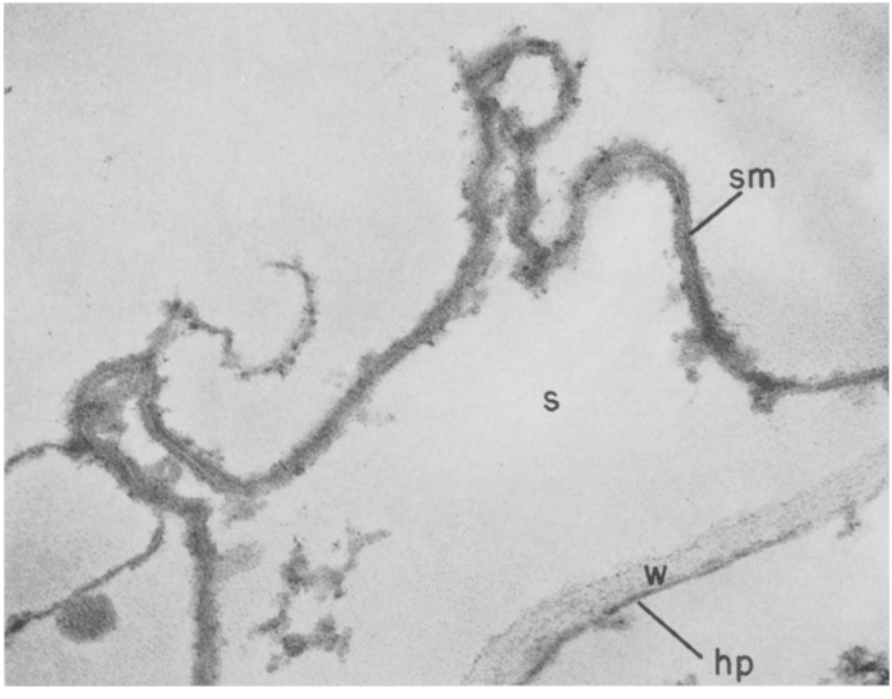

FIG. 8. Protrusions of the sheath membrane. Stained with lead citrate. Magnification × 80,000.

Uitstulpingen van de schedemembraan. Kleuring met loodcitraat. Vergroting 80.000 ×.

Other functions of the isolated haustoria are not yet known, but these studies warrant additional electronmicroscopic and metabolic investigations of the isolated haustoria since they may contribute to the knowledge of obligate parasitism.

SAMENVATTING

Er werd elektronenmicroscopisch onderzoek verricht aan haustoriën, geïsoleerd uit komkommerbladen geïnfecteerd met echte meeldauw *(Sphaerotheca fuliginea)*.

De haustoriën bevatten een centraal lichaam van 5 tot 7 μ met veel mitochondriën dat omgeven is door een schede van maximaal 4 μ die de elektronenbundel gemakkelijk doorlaat. De schede wordt begrensd door een geïnvagineerde membraan van ongeveer 0,03 μ.

Het centrale lichaam is via kronkelend verlopende uitstulpingen verbonden met de schedemembraan.

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