Neth. J. Pl. Path. 75 (1969) 169–177

The ultrastructure of powdery mildew, Sphaerotheca fuliginea, isolated from cucumber leaves

H. M. DEKHUIJZEN and CHRISTINA VAN DER SCHEER

Institute for Organic Chemistry TNO, Utrecht[1]
Service Institute for Technical Physics in Agriculture, Wageningen

Accepted 25 July, 1968

Abstract

The fine structure of conidia, hyphae, and of isolated haustoria of cucumber mildew (*Sphaerotheca fuliginea*) was studied. Large vacuoles are present in the spores and hyphae. Rosette-shaped particles, presumably glycogen, occur in the spores. Certain haustoria were found which lack the protrusions of the central body of mature haustoria. Most probably these haustoria are young and not yet fully developed. Portion of the sheath membrane of these haustoria is in close contact with the central body wall. The observations suggest that the haustorial sheath membrane is formed as a specialized portion of the haustorial body wall. Presumably the formation of the sheath membrane precedes the formation of the sheath and that of the protrusions of the central body.

Introduction

Spores are the only part of mildew and rust fungi which are readily available for the study of the mode of action of fungicides and systemic compounds on obligate parasites and for the study of relationships between hosts and obligate parasites. Recently, however, a method has been described for the isolation of metabolically active haustoria and hyphae from cucumber leaves infected with powdery mildew, *Sphaerotheca fuliginea* (Dekhuijzen, 1966). This method appeared also suitable for the isolation of bean rust hyphae (Dekhuijzen et al., 1967; Dekhuijzen and Staples, 1968).
Several workers studied the haustorial morphology in an effort to understand better the relationship between host and parasite (Bracker, 1967, 1968). Light microscope and electron microscope studies (Hirata, 1937, 1967; McKeen et al., 1966; Dekhuijzen and van der Scheer, 1967) showed that, similar to the formation of finger-like protrusions on the central body of *Erysiphe graminis*, protrusions emerge from the central body of cucumber powdery mildew haustoria. In case of *E. graminis* the protrusions extend straight over relatively large distances, whereas the protrusions of *S. fuliginea* grow very convolutely. Further electron microscope studies on isolated cucumber mildew haustoria showed, however, that protrusions do not always occur. This type of haustoria will be described more in detail and compared with the ultra-structure of conidia and of hyphae of cucumber powdery mildew.

[1] The study has been carried out at the Laboratory of Phytopathology, State Agricultural University, Wageningen, in connection with the activities of TNO Research Unit for Internal Therapy of Plants.

Materials and methods

Haustoria, conidia and hyphae of *S. fuliginea* were isolated, fixed in glutaraldehyde and osmium tetroxide, cut and stained with lead citrate and in a few cases also with uranyl acetate as described in previous papers (Dekhuijzen, 1966; Dekhuijzen and van der Scheer, 1967).

Results

The haustoria

Fig. 1 shows a section of an isolated haustorium with a large ellipsoidal haustorial body of about 3–6 μ. The body is limited by a wall of about 0.15 μ thickness and contains many mitochondria. Vacuoles varied in size and frequently contained electron-dense material and large bodies of unknown nature (Fig. 1, 2 and 3). This material is not an artifact due to the isolation procedure because it has also been found in vacuoles of haustoria which were not separated from the host cells (McKeen et al., 1966).
The haustorial body is surrounded by an electron lucent sheath which is limited by a heavily invaginated sheath membrane. Large host particles up to 0.5 μ diamater can be enveloped by the sheath membrane (Fig. 4, 5). The most surprising fact of Fig. 1 is the absence of protrusions on the haustorial body. Portions of the protrusions might have been missed during cutting but four serial sections of the same haustorium gave no indication of the presence of protrusions. Moreover, it is remarkable that a large portion of the sheath membrane is in close contact with the haustorial body (Fig. 2, 3). It is well known that the haustorial sheath of powdery mildews and rusts increases with the age of the haustorium (H. G. Ehrlich and M. A. Ehrlich, 1963; Shaw and Manocha, 1965; McKeen et al., 1966; Hirata, 1967; Bracker, 1967). The haustorial age could not

Key

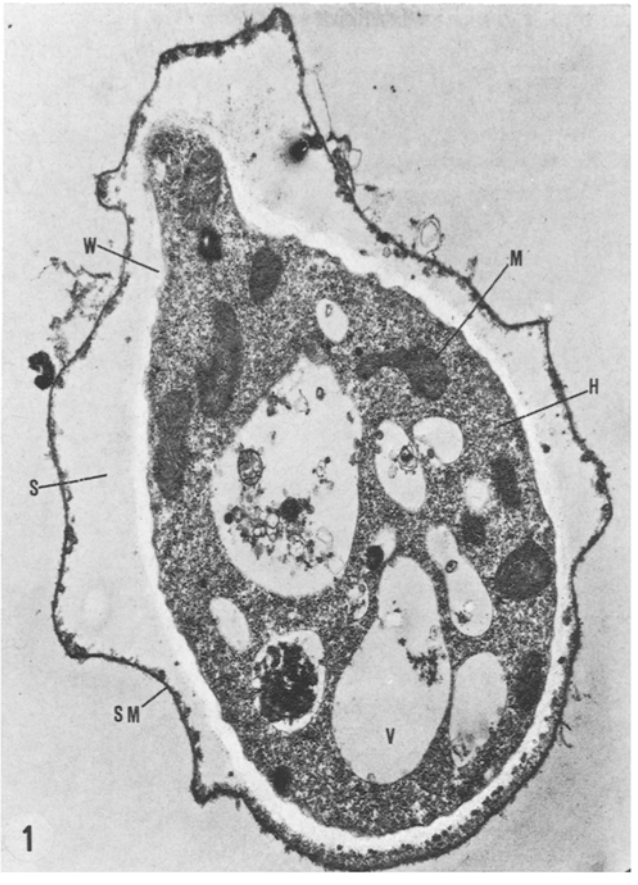
G = glycogen; H = haustorial body; HP = haustorial plasma membrane; I = invagination; M = mitochondrion; N = nucleus; P = protrusion of haustorial body; S = sheath; SE = septum; SM = sheath membrane; V = vacuole; W = wall of haustorial body, of conidium, or of hypha.

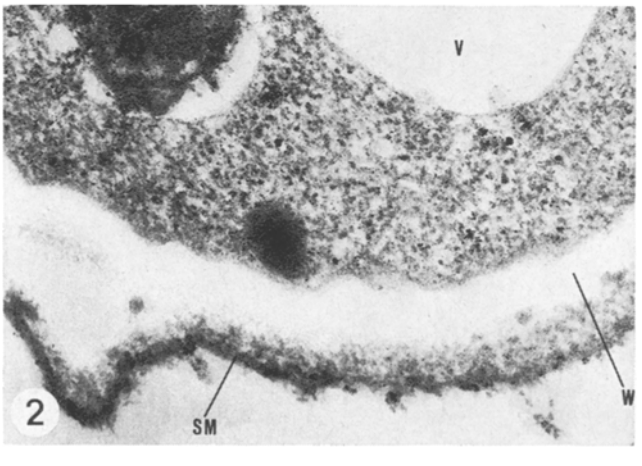
Afkortingen

G = glycogeen; H = centrale lichaam; HP = membraan van het centrale lichaam; I = invaginatie; M = mitochondrium; N = kern; P = uitstulping van het centrale lichaam; S = schede; SE = septum; SM = schedemembraan; V = vacuole; W = wand van het centrale lichaam, van het conidium of van de hyphe.

Fig. 1. A section of an isolated haustorium of *Sphaerotheca fuliginea* stained with lead citrate. Magnification × 16,000.
Fig. 2. Detail of Fig. 1 showing vacuoles with large dense bodies and portion of the sheath membrane which is in close contact with the haustorial body wall. Magnification × 60,000.

Fig. 1. Een doorsnede door een geïsoleerd haustorium van Sphaerotheca fuliginea, gekleurd met loodcitraat. Vergroting 16.000 ×.
Fig. 2. Detail van Fig. 1 met vacuolen waarin grote donkere lichamen aanwezig zijn en met een schedemembraan dat in nauw contact staat met de wand van het centrale lichaam. Vergroting 60.000 ×.


W
M
H
S
SM
V
1


V
W
SM
2

be determined accurately, but Fig. 1 might be interpreted as a section of a young haustorium in which the sheath membrane is being withdrawn from the body wall. Most probably this stage precedes the stage in which the protrusions of the central body are formed (Fig. 3).

The conidia

The conidium is surrounded by a wall which is composed of an inner layer with a thickness of about 0.2 μ and an outer layer of about the same size (Fig. 6, 7). Large vacuoles are present which occupy most of the spore volume. Mitochondria and rosette shaped granules (Fig. 8), similar to those depicted for the conidia of *E. cichoracearum* by McKeen et al., (1967), are visible in the cytoplasm. The rosette shaped granules may be glycogen granules because of their resemblance to glycogen particles in animal tissue and fungi (Foerster et al., 1965; Bracker, 1967).

The hyphae

Fig. 9 depicts a longitudinal section of a hyphal fragment isolated from the host. The fungal cell wall has a thickness of about 0.15 μ and consists of two different zones. The two septae separate the cytoplasm into three distinct portions, viz. a portion which contains only remnants of cytoplasm, and two portions with dense cytoplasm and large vacuoles. A nucleus is visible in the central segment. The three portions may represent three different stages in the hyphal growth, a senescent, a mature and a young developing one.

Discussion

The presence of glycogen in fungi is a well established fact (Cochrane, 1958). This storage polysaccharide occurs as rosette shaped particles as determined by electron microscopy and cytochemical tests (Foerster et al., 1957). So far, however, there is no chemical evidence for the occurrence of glycogen in powdery mildew spores.

The electron microscope study of McKeen et al. (1967) showed also the presence of large vacuoles in powdery mildew spores. These results are consistent with the high

Fig. 3. Isolated haustorium stained with lead citrate. Magnification × 17,000.

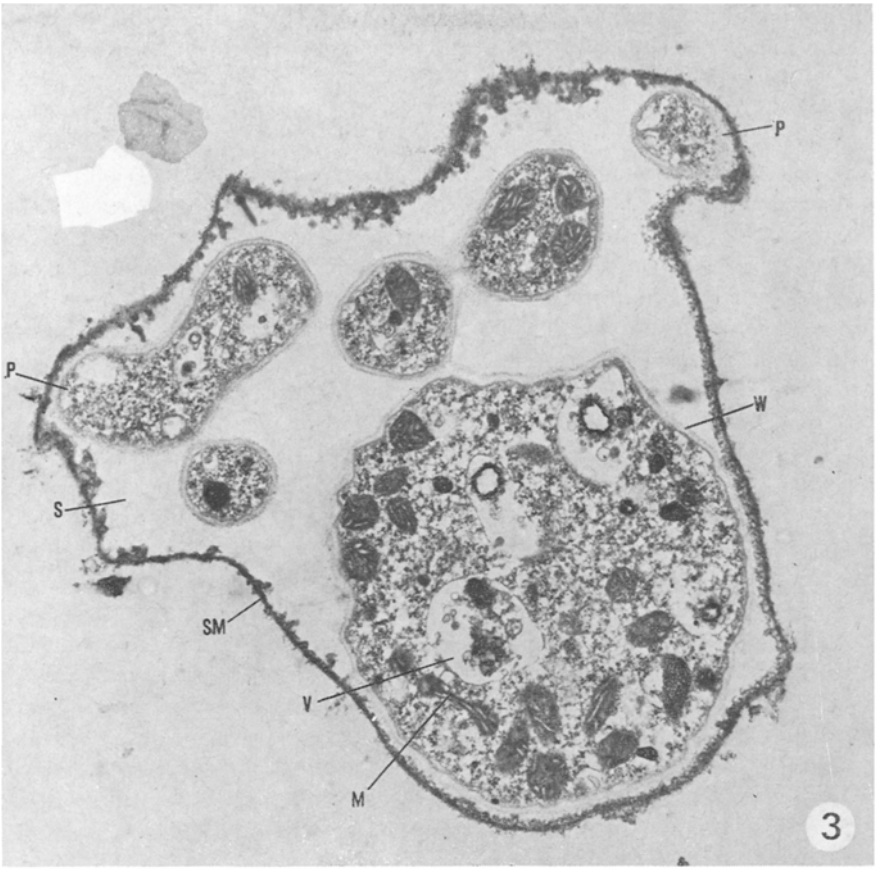
Fig. 4. A large particle of host material enveloped by the sheath membrane of a mature haustorium and stained with lead citrate. Magnification × 45,000.

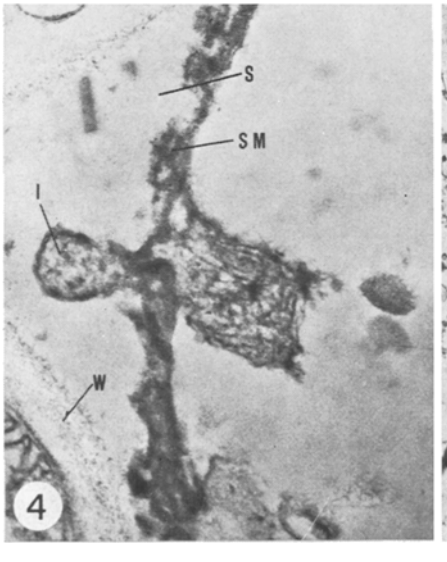
Fig. 5. Invagination of sheath membrane of a mature haustorium stained with lead citrate. Magnification × 23,000.

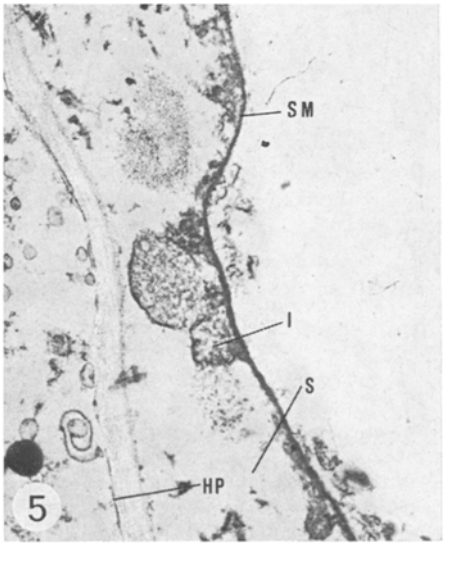
Fig. 3. Geïsoleerd haustorium gekleurd met loodcitraat. Vergroting 17.000 ×.

Fig. 4. Waardplant materiaal omhuld door de schedemembraan van een volwassen haustorium. Kleuring met loodcitraat. Vergroting 45.000 ×.

Fig. 5. Invaginatie van de schedemembraan van een volwassen haustorium gekleurd met loodcitraat. Vergroting 23.000 ×.


P
P
W
S
SM
V
M
3


S
SM
I
W
4


SM
I
S
HP
5

water content of powdery mildew spores as found by Yarwood (1950) and explain the ability of these spores to germinate at low humidities. Large vacuoles were also observed in the hyphae.

A dense cytoplasm was not found in every hyphal segment. A similar phenomenon occurs in isolated bean rust hyphae (Dekhuijzen et al., 1967). Whether cytoplasm degenerates in segments which are growing older or whether it has been withdrawn into new formed cell segments, as has been found with *Basidiobolus ranarum* (Robinow, 1963), remains a matter of speculation .The presence of the senescent segments in the hyphae explains also the fact that the oxygen uptake of isolated hyphae based on dry weight was found to be five times lower than of the spores (Dekhuijzen, 1966).

As has been shown by Bracker (1968) the hyphal wall of barley powdery mildew extends through the host cell wall to the haustorial body wall. The opinion on the origin of the sheath and the sheath membrane remains a matter of conflict. H. G. Ehrlich and M. A. Ehrlich (1963) proposed that the sheath membrane of *E. graminis* is not merely the host ectoplast but is a membrane at least partly of fungal origin. Bracker (1967, 1968) considers the sheath membrane as a specialized portion of the host plasma membrane and the sheath as the zone between haustorium and host protoplast. The following facts, however, are difficult to reconcile with Bracker's view. First, studies on isolated haustoria have shown that the sheath membrane can easily be separated from the host plasma membrane but is firmly attached to the sheath or to the haustorial body wall (Fig. 1, 3). Secondly, it is difficult to conceive of a sheath membrane formed by the host but which is actively engaged in the envelopment of large host particles (Fig. 4) leading to a nutrition of the parasite. Such large host particles were never observed to pass the haustorial body wall. It is conceivable that these particles are first broken down into smaller units by enzymes in the sheath prior to absorption by the central body wall.

Fig. 6. Section of a portion of a conidium of cucumber powdery mildew stained with lead citrate. Magnification × 15,000.

Fig. 7. Detail of Fig. 6. Magnification × 60,000.

Fig. 8. Portion of a conidium with glycogen particles. Stained with lead citrate. Magnification × 18,000.

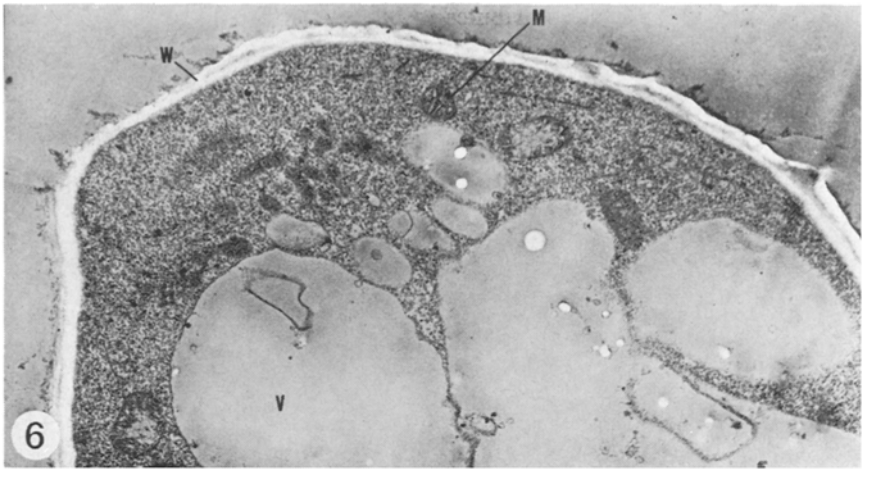
Fig. 9. Longitudinal section of a hypha of cucumber powdery mildew isolated from cucumber leaves and stained with uranyl acetate and lead citrate. Magnification × 5000. Inset, part of hyphal wall at a magnification of × 37,000.

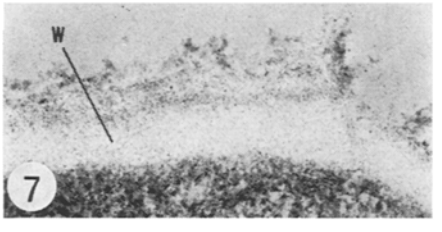
Fig. 6. Doorsnede van een gedeelte van een conidium van echte komkommermeeldauw. Kleuring met loodcitraat. Vergroting 15.000 ×.

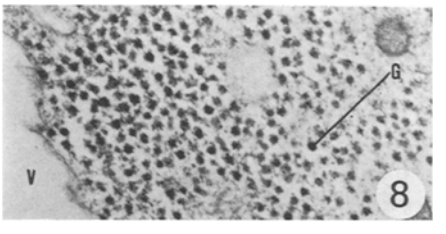
Fig. 7. Detail van Fig. 6. Vergroting 60.000 ×.

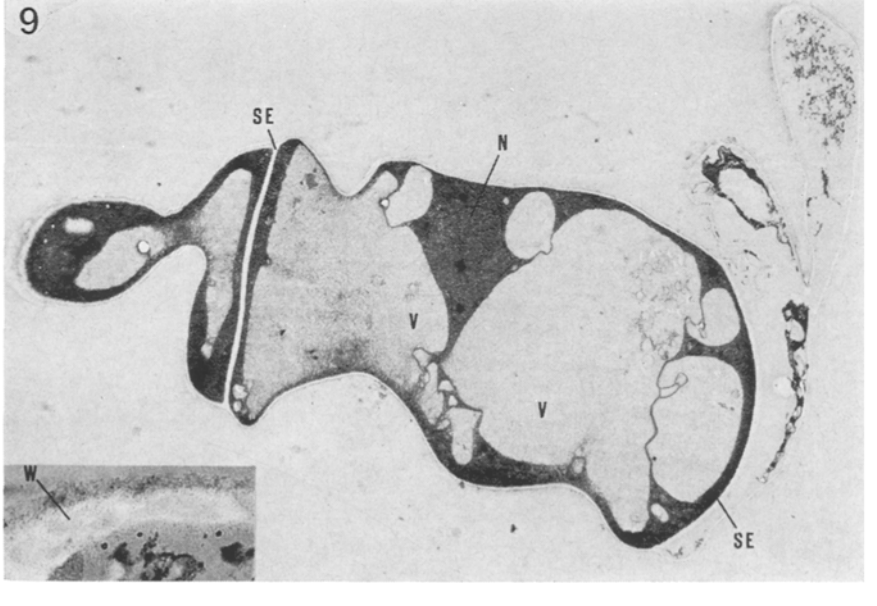
Fig. 8. Gedeelte van een conidium met glycogeen. Kleuring met loodcitraat. Vergroting 18.000 ×.

Fig. 9. Lengtedoorsnede van een hyphe van echte komkommermeeldauw, geïsoleerd van een komkommerblad. Kleuring met uranylacetaat en loodcitraat. Vergroting 5000 ×. Inzet: een gedeelte van de hyphewand bij een vergroting van 37.000 ×.


M
W
V
6


W
7


G
V
8


9
SE
N
V
V
W
SE

The possibility that the host contributes in the formation of the sheath membrane cannot be excluded but the micrographs presented here rather suggest that the haustorial sheath membrane is formed as a specialized portion of the haustorial body wall. Subsequently the sheath might be formed by secretion of an amorphous electron-lucent liquid which is most probably of fungal origin. Presumably the sheath membrane draws away from the body wall during the formation of the sheath. Shortly after formation of the sheath, or already during its formation, protrusions emerge from the central body. The sheath may contain enzymes for the breakdown of host material which is enveloped by the sheath membrane. Further electron microscope studies and localization of enzyme activities are required to obtain more evidence for this view.

Samenvatting

De submicroscopische structuur van echte meeldauw, Sphaerotheca fuliginea, geïsoleerd uit komkommerbladeren

Er werd een onderzoek verricht naar de submicroscopische structuur van conidia, hyphen en haustoriën, geïsoleerd uit komkommerbladeren die geïnfecteerd waren met echte meeldauw (*Sphaerotheca fuliginea*). Sporen en hyphen bevatten grote vacuolen. In de conidiën werden rozetachtige deeltjes, waarschijnlijk glycogeen, gevonden. Er werden ook haustoriën gevonden, waarbij de normaal voorkomende uitstulpingen van het centrale lichaam in volwassen haustoriën ontbraken. Bij deze waarschijnlijk jonge haustoriën is een gedeelte van de schedemembraan in nauw contact met de wand van het centrale lichaam. Deze resultaten doen vermoeden dat de schedemembraan gevormd wordt als een speciaal gedeelte van de wand van het centrale lichaam. Waarschijnlijk scheidt de membraan zich daarna van het centrale lichaam bij de vorming van de schede. Verondersteld wordt, dat de uitstulpingen van het centrale lichaam nà of tijdens de vorming van de schede ontstaan.

References

Bracker, C. E., 1967. Ultrastructure of fungi. A. Rev. Phytopath. 5: 343–374.

Bracker, C. E., 1968. The ultrastructure of the haustorial apparatus of Erysiphe graminis and its relationship to the epidermal cell of barley. Phytopathology 58: 12–30.

Cochrane, V. W., 1958. Physiology of fungi. Wiley, New York.

Dekhuijzen, H. M., 1966. The isolation of haustoria from cucumber leaves infected with powdery mildew. Neth. J. Pl. Path. 72: 1–11.

Dekhuijzen, H. M. and Scheer, C. van der, 1967. Electron microscopic observations on haustoria isolated from cucumber leaves infected with powdery mildew. Neth. J. Pl. Path. 73: 121–125.

Dekhuijzen, H. M., Singh, H. and Staples, R. C., 1967. Some properties of hyphae isolated from bean leaves infected with the bean rust fungus. Contr. Boyce Thompson Inst. Pl. Res. 23: 367–372.

Dekhuijzen, H. M. and Staples, R. C., 1968. Mobilization factors in uredospores and bean leaves infected with bean rust fungus. Contr. Boyce Thompson Inst. Pl. Res. 24:39-52.

Ehrlich, H. G. and Ehrlich, M. A., 1963. Electronmicroscopy of the sheath surrounding the haustorium of Erysiphe graminis. Phytopathology 53: 1378–1380.

Foerster, G. E., Behrens, P. Q. and Airth, R. L., 1965. Bioluminescence and other characteristics of Collybia velupites. Am. J. Bot. 52: 487–495.

Hirata, K., 1937. The haustoria of some powdery mildews. Ann. phytopath. Soc. Japan 6: 319–334.

Hirata, K., 1967. Notes on haustoria, hyphae and conidia of the powdery mildew fungus of barley, Erysiphe graminis f. sp. hordei. Mem. Fac. Agric. Niigata Univ. 6: 207–257.

McKeen, W. E., Smith, R. and Mitchell, N., 1966. The haustorium of Erysiphe cichoracearum and the host–parasite interface on Helianthus annuus. Can. J. Bot. 44: 1299–1306.
McKeen, W. E., Mitchell, N. M. and Smith, R., 1967. The Erysiphe cichoracearum conidium. Can. J. Bot. 45: 1489–1496.
Robinow, C. F., 1963. Observations on cell growth, mitosis and division in the fungus Basiobolus ranarum. J. Cell. Biol. 17: 123–152.
Shaw, M. and Manocha, M. S., 1965. The physiology of host–parasite relations. XV. Fine structure in rust-infected wheat leaves. Can. J. Bot. 43: 1285–1292.
Yarwood, C. E., 1950. Water content of spores. Am. J. Bot. 37: 636–639.