# Fine-Structure Analysis of Host-Parasite Relations in the Spot Anthracnose of Desmodium

David L. Mason and Charles L. Wilson

Associate Professor, Department of Biology, Wittenberg University, Springfield, OH 45501; and Research Plant Pathologist, Agricultural Research Service, U. S. Department of Agriculture (USDA) and Adjunct Professor, Ohio State University, Columbus, OH 45501. Address of second author: Agricultural Research Service USDA, North Central Region, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, OH 44601.

Research was supported by National Institutes of Health Grant No. AI 11556-01 from the National Institute of Allergy and Infectious Diseases.

We thank Jeanie Jo Wester for her help in preparing the manuscript.



Accepted for publication 6 July 1977.

ABSTRACT

MASON, D. L., and C. L. WILSON. 1978. Fine structure analysis of host-parasite relations in the spot anthracnose of Desmodium. Phytopathology 68:65-73.

The pathogen *Elsinöe wisconsinensis* grows intercellularly in *Desmodium illinoense*. Host cells in close association with the pathogen undergo extensive hypertrophy and hyperplasia. The only cells destroyed are the epidermal cells which become enveloped by mycelia and crushed by the developing stroma. Chloroplasts in parasitized cells are smaller than normal, contain fewer grana and thylakoids per granum, and they generally lack starch granules. The number of Golgi bodies and Golgi vesicles are more abundant, and they reside in those parts of host cells associated with hyphae. Pinocytic vesicles containing a granular to fibrillar material occur abundantly in parasitized cells. These vesicles appear to pass through the hyaloplasm, and become enclosed in a second membrane originating from the tonoplast. They ultimately become deposited in the central vacuole, where they are broken down, and their contents are digested. Golgi vesicles fuse with newly formed and migrating pinocytic vesicles, the plasmalemma, and the tonoplast. The uninucleate intercellular fungal cells contain most of the organelles common to eucaryotes. Spherosome contents and glycogen granules are present in the fungal cell vacuoles, where they appear to be digested.

Few attempts have been made to ascertain the relationships between the mycelia of a strictly intercellular parasite and associated host cells. The spot anthracnose of *Desmodium illinoense* Gray, incited by *Elsinöe wisconsinensis* Greene, involves such a relationship. An earlier light-microscopic study provided insight into the nature of host-parasite relations in this disease (7). the present study primarily was made to determine the fine-structure relationship that exists between the fungus and its host.

## MATERIALS AND METHODS

In order to follow the early stages of infection, healthy plants of *D. illinoense* grown in the greenhouse from seed were inoculated by spraying with a water suspension of spores and mycelial fragments of *E. wisconsinensis*. The suspension was made by grinding freshly picked infected leaves and stems in distilled water in a blender. The resulting slurry was strained through four layers of cheesecloth. Diseased plants used as a source of inoculum were collected from the University of Wisconsin Arboretum, Madison, Wisconsin, in June of 1972. Typical lesions appeared on the stems and leaves about 4 wk later.

Both healthy and infected stem and leaf segments were prepared in various ways for electron miscroscopy. They were fixed at room temperature for 3-5 hr, using 3% glutaraldehyde in 0.05 M phosphate buffer at pH 6.8. The material was washed for 1 hr in four changes of 0.05 M phosphate ($K^+$) buffer, postfixed in 2% osmium tetroxide in 0.05 M phosphate buffer for 2 hr, dehydrated in an acetone series, and embedded in Araldite-Epon (9): sections were mounted on [48 × 229 μm (300 × 75-mesh), 58% open space] grids, stained with 2% uranyl acetate followed by lead citrate (11), and examined with a Zeiss EM 9 electron microscope operating at 45 kV.

## OBSERVATIONS

Although the pathogen *E. wisconsinensis* is capable of invading any organ of *D. illinoense*, this study involved only infected leaves and stems. Evidence obtained from light microscopy indicated that the fungus penetrated between epidermal cells in a manner similar to that described by Fitzpatrick (3) for leaf invasion by

0032-949X/78/000010 $03.00/0

*Taphrina deformans* (Berk.) Tul. Once beneath these cells, the hyphae continued to grow intercellularly in the epidermal and subepidermal regions. Branches from the initial filaments in the leaf blade and older stem infections grew outward and encased the epidermal cells (Fig. 1). At the same time, other branches extended inward between the subepidermal cells (Fig. 3, 6) and induced extensive hypertrophy and hyperplasia (Fig. 3, 6). The invading hyphae grew exclusively between the host cells, and no cellular penetration was observed.

Cellular modifications occurred only in host cells in close proximity to a hypha. In the leaf, the most pronounced modifications occurred in the palisade and spongy mesophyll cells; in the stem, they were restricted to the cortical cells. Electron micrographs of both healthy (Fig. 2) and diseased cortical cells (Fig. 3, 6) clearly demonstrate the extensive morphological modifications of host cells. Although somewhat altered, these subepidermal cells retained their integrity and appeared to remain alive throughout the normal life of the plant.

From light microscopic observations, it appeared that the fungus grew just beneath the cuticle. Electron microscopic studies showed, however, that the pathogen grew within the cell walls beneath the cuticle (Fig. 4). Apparently, hyphal invasion within the host wall was by mechanical disruption since there was no indication of enzymatic degradation. The epidermal cells were not destroyed immediately by the presence of the enveloping mycelia, as evidenced by clearly defined nuclei (Fig. 1).

The fungus continued to develop within the outer walls and between the epidermal cells. As this occurred, the epidermal cells became crushed and destroyed apparently by a purely mechanical process. Gradually, an organized stroma developed on the surface of the diseased organ. The remaining walls of crushed epidermal cells frequently were observed in the basal part of a stroma (Fig. 5).

The most pronounced host cell modifications occurred in the subepidermal cells. The most obvious differences between normal and parasitized subepidermal cells were their size and morphology. Normal cortical cells were fairly uniform in size and shape and were partially separated by intercellular spaces (Fig. 2). Parasitized cortical cells typically were larger and highly irregular in shape; intercellular spaces were absent (Fig. 3, 6). The enlarged cortical cells frequently appeared to have compartmentalized into smaller internal cellular units (Fig. 6). The walls of each cellular compartment were somewhat irregular and thin (Fig. 6). The same type of hypertrophic and hyperplastic modification also was frequently observed in leaf mesophyll cells.

Certain cellular organelles were modified in structure and number within diseased host cells. Although diseased cells contained well defined nuclei, chloroplasts typically were reduced in size and number. Sections through normal (Fig. 2) and proliferated (Fig. 3, 6) cortical cells showed these differences in the chloroplasts. It was difficult to account for the reduction in number, because there was no indication of breakdown of these organelles. As cell divisions occurred, the plastids became distributed among the newly forming cells. This was not always uniform because in infected leaves the modified palisade cells closest to the epidermal layer typically contained fewer chloroplasts than those adjoining the spongy mesophyll cells. A comparison of the chloroplasts in parasitized (Fig. 7) and healthy cells (Fig. 8) revealed that plastids in the former were smaller, contained fewer grana and fewer thylakoids per granum, and usually lacked starch.

Conversely, Golgi bodies were present in greater number in diseased than in healthy cells. These organelles were most abundant in that part of the cytoplasm where the host and fungus were in contact (Fig. 9). Large pinocytic vesicles (phagosomes) were found only in diseased cells. These vesicles appeared to originate as invaginations from the host plasmalemma (Fig. 9, 10, 16) in regions where the host and fungal cells were in close contact (Fig. 9). In some instances these invaginations (developing phagosomes), which contained a somewhat granular to fibrillar material, extended through the hyaloplasm and became enveloped by the tonoplast (Fig. 10). In other instances the phagosomes appeared in the hyaloplasm (Fig. 11, 16) or within invaginations of the tonoplast (Fig. 12, 16) or within the vacuole (Fig. 13, 16). In each case the vesicles had well-defined unit membranes, which had the same thickness as that of the plasmalemma (Fig. 11). Numerous Golgi vesicles were commonly observed in the vicinity of (Fig. 9, 14, 15, 17, 18), associated with (Fig. 9, 14, 15, 17, 18), and occasionally attached to pinocytic invaginations and pinocytic vesicles (phagosomes) (Fig. 9, 14, 15, 17, 19, 20, 21). Golgi vesicles also were observed near and touching the plasmalemma (Fig. 19) and tonoplast in parasitized cells (Fig. 22).

Electron microscopic observations revealed that the pathogen readily invaded the cortex of stems, but did not grow between the phloem fibers and into the vascular tissue. The thick-walled fiber cells, which have little or no intercellular spaces between them, appeared to be a physical barrier to the invading parasite (Fig. 23).

The invading intercellular hyphae of *E. wisconsinensis* were composed of short, rectangular cells. Each cell had a single nucleus, typically containing three to five dark-staining regions (Fig. 24). Other organelles commonly found in eucaryotic cells were observed (Fig. 24, 25, 26, 27, 28, 29, 30) including mitochondria, rough endoplasmic reticulum, spherosomes, microbodies, and vacuoles. Golgi bodies were not observed in the fungus. Microbodies occasionally contained a crystal (Fig. 25). Particles with the appearance of large coated vesicles commonly were detected in cells of the outer layers of the stroma (Fig. 26). The cells were separated by septa with well-defined septal pores, and Woronin bodies were typically found adjacent to the pores (Fig. 27). Spherosomes frequently were observed to be fused with the tonoplast (Fig. 28), or apparently discharging their contents into the vacuole (Fig. 29). Glycogen granules were observed in pinocytic vesicles originating from the tonoplast (Fig. 30). These vesicles appeared to be pinched off into the vacuole and there broken down to release the glycogen granules (Fig. 30) which appeared to be digested (Fig. 30).

## DISCUSSION

The hypertrophic and hyperplastic responses in host cells associated with intercellular hyphae suggest either that a growth hormone is secreted by *E. wisconsinensis* or

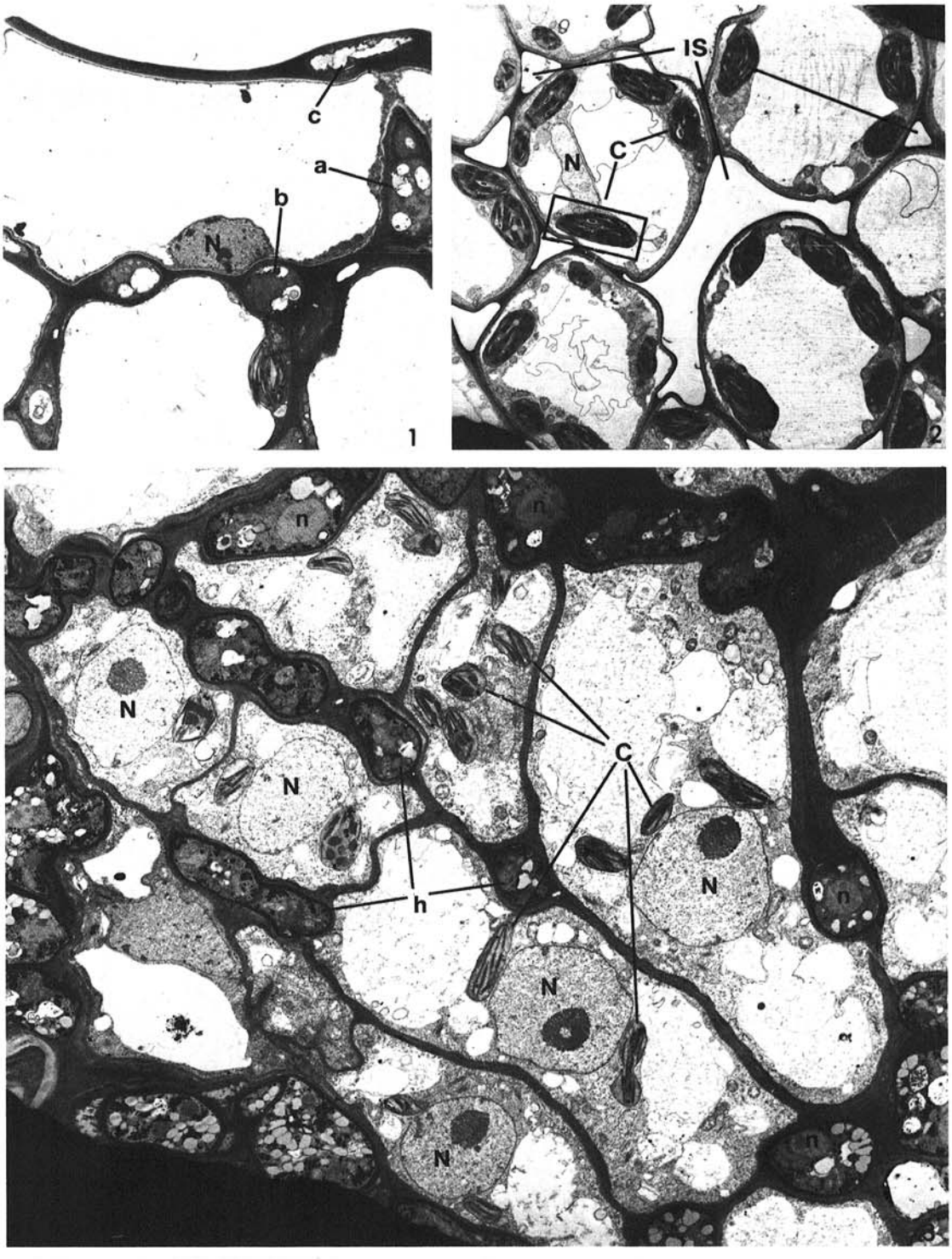


**Fig. 1-3.** Electron micrographs of cross sections through healthy and diseased (pathogen: *Elsinöe wisconsinensis*) tissues of *Desmodium illinoense*. **1)** Diseased leaf, showing fungal hyphae (a) between epidermal cells, (b) between epidermal and palisade cells, and (c) between wall layers of the epidermal cell. Note the host cell nucleus (N). × 2,500. **2)** Healthy stem, showing a nucleus (N) and chloroplasts (C) in cortical cells and intercellular spaces (IS). × 2,800. **3)** Diseased stem, showing fungal hyphae (h) between hypertrophic and hyperplastic cortical cells. Note the host cell nuclei (N), chloroplasts (C), and fungal cell nuclei (n). × 3,300.

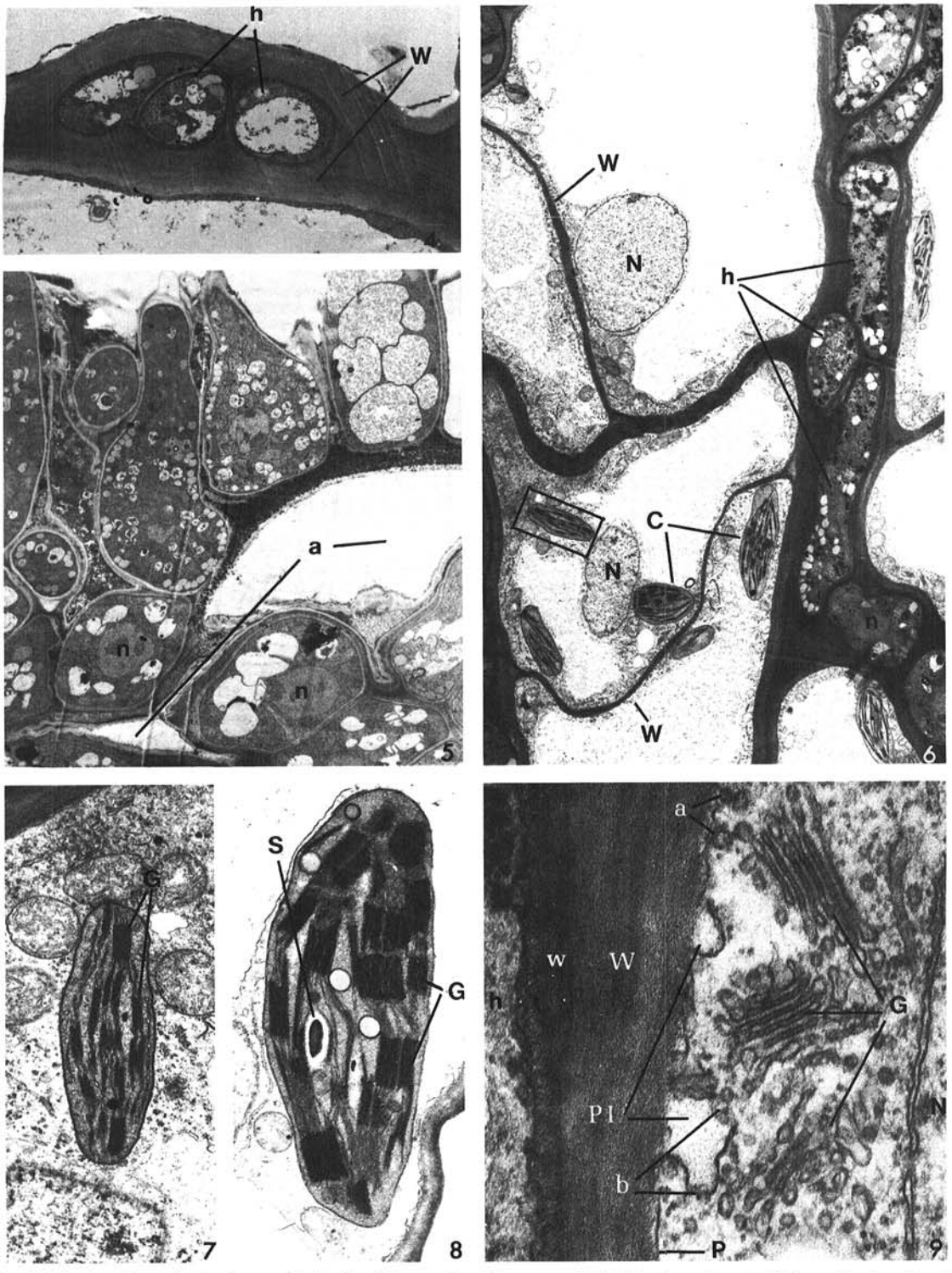


**Fig. 4-9.** Sections through closely associated cells of *Desmodium illinoense* and *Elsinöe wisconsinensis*. **4)** Fungal hyphae (h) within the wall (W) of an epidermal cell. × 3,600. **5)** A section through a developing fungal stroma, showing the remains of two partially crushed epidermal cells (a). Fungal nuclei (n). × 4,000. **6)** Hyphae (h) between proliferated stem cortical cells. The host cells have enlarged and divided into cellular compartments having thin and somewhat irregularly-shaped walls (W). Note the host cell nucleus (N) and chloroplasts (C) which are smaller than those in healthy cortical cells, and fungal cell nuclei (n). × 3,600. **7)** An enlargement of a chloroplast outlined in Fig. 6. The plastid is smaller, and it contains fewer thylakoids per granum (b) than the typical plastid in a nonparasitized cell shown in Fig. 8. × 13,000. **8)** An enlargement of a healthy chloroplast outlined in Fig. 2. The grana (G) are well-defined, and a starch granule (S) is seen. × 11,000. **9)** Fungal hyphae (h) and host cell in direct contact, showing Golgi bodies (G), pinocytic invaginations (PI) (developing phagosomes), and Golgi vesicles (GV) within the host cell. Golgi vesicles appear to be closely associated with and contacting a pinocytic invagination (b) and the plasmalemma (a). Note the host cell wall (W), plasmalemma (P), and nucleus (N), and the fungal hyphae (h) and wall (w). × 51,000.

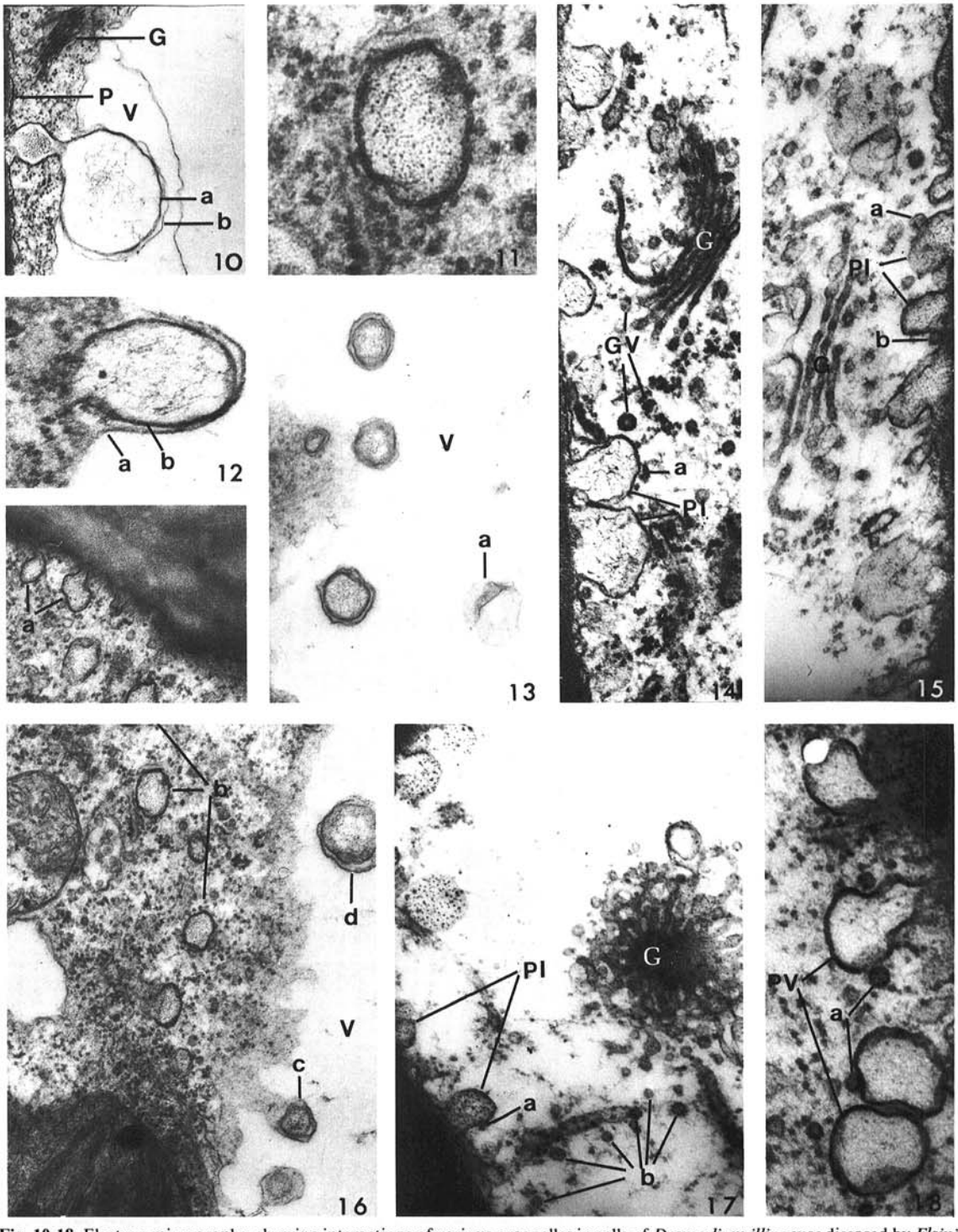


**Fig. 10-18**. Electron micrographs, showing interactions of various organelles in cells of *Desmodium illinoense* diseased by *Elsinöe wisconsinensis*. **10**) A pinocytic invagination (developing phagosome), extending through the hyaloplasm, partially encased by the tonoplast of the host vacuole (V). The membrane of the invagination (a) is an extension of the host plasmalemma (P), and it is thicker than the tonoplast (b). Note the closely associated Golgi body (G) and the granular to fibrillar material within the developing phagosome. × 20,000. **11**) An enlargement of a portion of Fig. 16, showing a phagosome containing a granular material. The membrane of the phagosome is the same thickness as that of the plasmalemma. × 96,000. **12**) A phagosome (b) partially encased by the tonoplast (a). × 80,000. **13**) Double membrane-bound phagosomes within the vacuole (V). The one at "a" appears to be breaking down. × 18,000. 14-15) Host cells showing Golgi bodies (G), Golgi vesicles (GV), and pinocytic invaginations (PI). **14**) × 47,000. **15**) × 47,000. The Golgi vesicles appear to be closely associated with pinocytic invagination (developing phagosome), and in each figure one is touching a pinocytic invagination (a), and Figure 15 one is touching the plasmalemma (b). **16**) A series of vesicles (phagosomes) within the hyaloplasm and the vacuole. At "a" the plasmalemma is invaginated giving rise to two pinocytic invaginations (developing phagosomes); at "b" three phagosomes can be seen in the hyaloplasm; at "c" a phagosome is partially encased by the tonoplast; at "d" a phagosome, double membrane bound, is seen within the vacuole (V). × 32,000. **17**) A section, showing pinocytic vesicles (PV) (phagosomes), a Golgi body, (G) and Golgi vesicles (b). Note that the Golgi vesicles at "b" are arranged in a series between the Golgi body and plasmalemma, and that a Golgi vesicle (a) appears to be attached to a pinocytic invagination (PI). × 35,000. **18**) Golgi vesicles (a) closely associated with pinocytic vesicles (PV) (phagosomes). × 50,000.

that the pathogen stimulates the host to produce hormones which in turn induce the abnormal growth responses. The pronounced cellular modifications and host-parasite relations that occur in peach leaves infected by *Taphrina deformans* (3, 6) are very similar to those occurring in the spot anthracnose of *Desmodium*. It has been demonstrated in culture that *T. deformans* produces auxin (5) which may be responsible for eliciting the abnormal growth in diseased peach leaves.

Two features that were difficult to explain were the reduction in number and nonuniform distribution of chloroplasts in diseased host cells. There was no ultrastructural evidence that chloroplasts were being broken down. It appeared that the host cells enlarged and divided, but the chloroplasts did not divide, or they divided at a reduced rate. Counts made on the number of chloroplasts in diseased palisade cells lend support to this suggestion. Palisade cells in association with intercellular

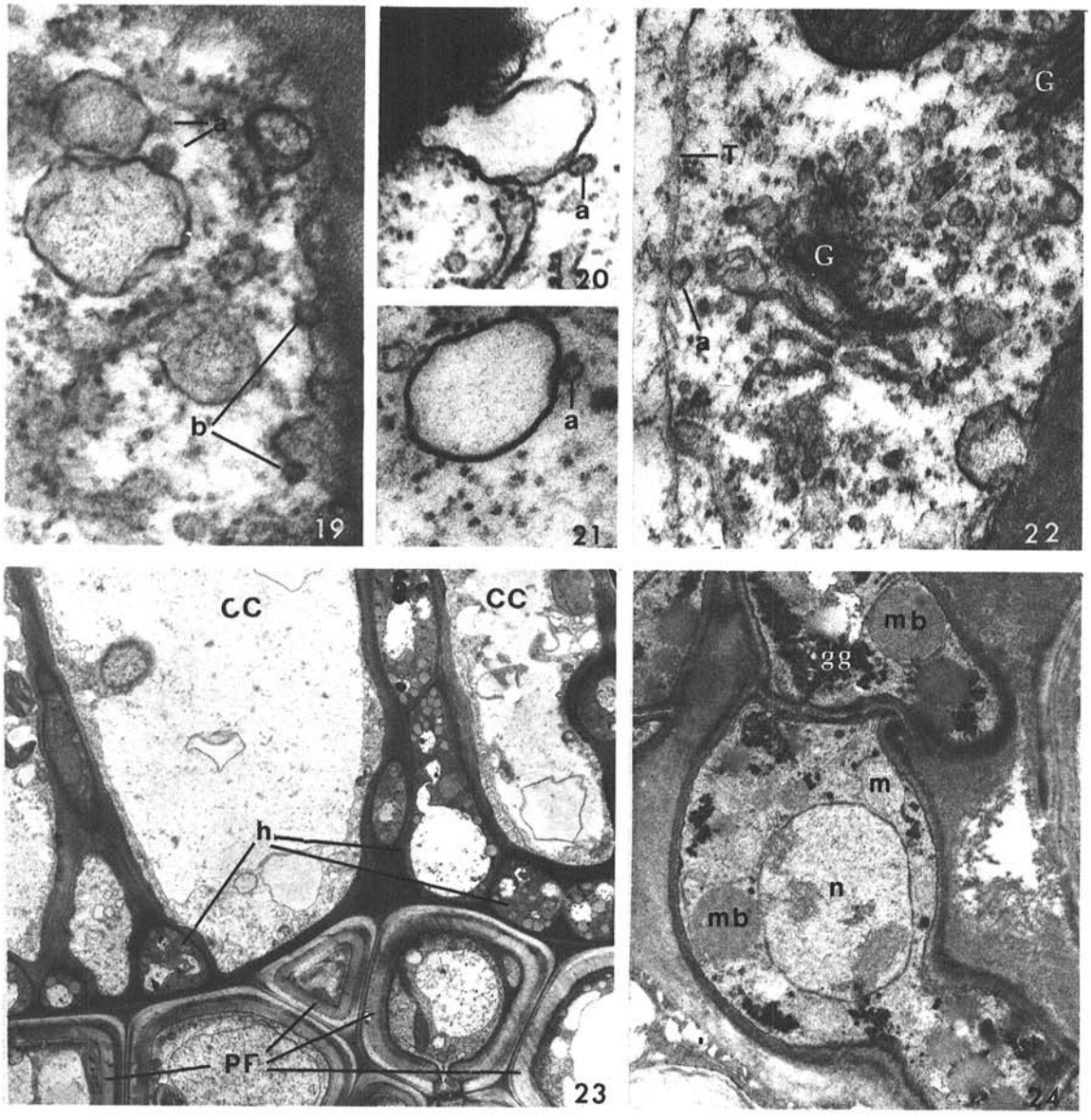


**Fig. 19-24.** Spot anthracnose of *Desmodium illinoense* caused by *Elsinöe wisconsinensis*. Electron micrographs, showing interactions of various organelles in diseased cells of *D. illinoense*, host-parasite interactions, and the fine-structure of *E. wisconsinensis* cells. **19)** Diseased host cells, showing Golgi vesicles (a) attached to pinocytic vesicles and to the plasmalemma (b). × 62,000. **20)** A Golgi vesicle (a) attached to a pinocytic invagination of the plasmalemma. × 60,000. **21)** A Golgi vesicle (a) attached to a pinocytic vesicle. × 60,000. **22)** A Golgi vesicle (a) associated with the tonoplast (T). Note the Golgi body (V) × 45,000. **23).** Fungal hyphae (h) between proliferated stem cortical cells (CC). Note that the hyphae do not extend between the phloem fibers (PF) cells. × 2,800. **24)** Fungal cells, containing a mitochondrion (m), glycogen granules (gg), nucleus (n), and microbody (mb). × 14,500.

hyphae appear to elongate and divide transversely to form stacks of brick-like cells. [See micrographs in (7)]. The total number of chloroplasts in such a stack of cells closely approximates that in a healthy palisade cell. Distribution of chloroplasts was not always uniform; within the stack the innermost cells associated with the spongy mesophyll layer contained a greater number of chloroplasts than those just beneath the epidermal layer. This unequal distribution could be accounted for if cell divisions always occurred in the outermost cell in a forming stack. Consequently, the number of plastids in the outermost cells would always be less than in those below.

Light microscopic evidence indicated that the fungus grew just beneath the cuticle (7). However, electron micrographs of diseased tissues show hyphae between the outer wall layers of the epidermal cells. It appeared that growth between these layers and between host cells in general was purely mechanical and not enzymatic. No enzymatic degradation of host cell wall materials was observed at hyphae tips. Additional evidence in support of purely mechanical intercellular growth comes from observations of growth of hyphae in the stem cortex. The pathogen readily grew inward between the cortical cells, but once reaching the densely packed, thick-walled phloem fibers, hyphae appeared to be unable to force their way between these cells into the vascular tissue.

Of interest in this study is the origin and fate of the rather large pinocytic vesicles and their interactions with other vesicles and organelles in diseased host cells. From numerous observations of electron micrographs, we have developed an interpretation of the interactions taking place among the various vesicles and organelles (Fig. 31). Pinocytic vesicles (phagosomes) containing a fibrillar to

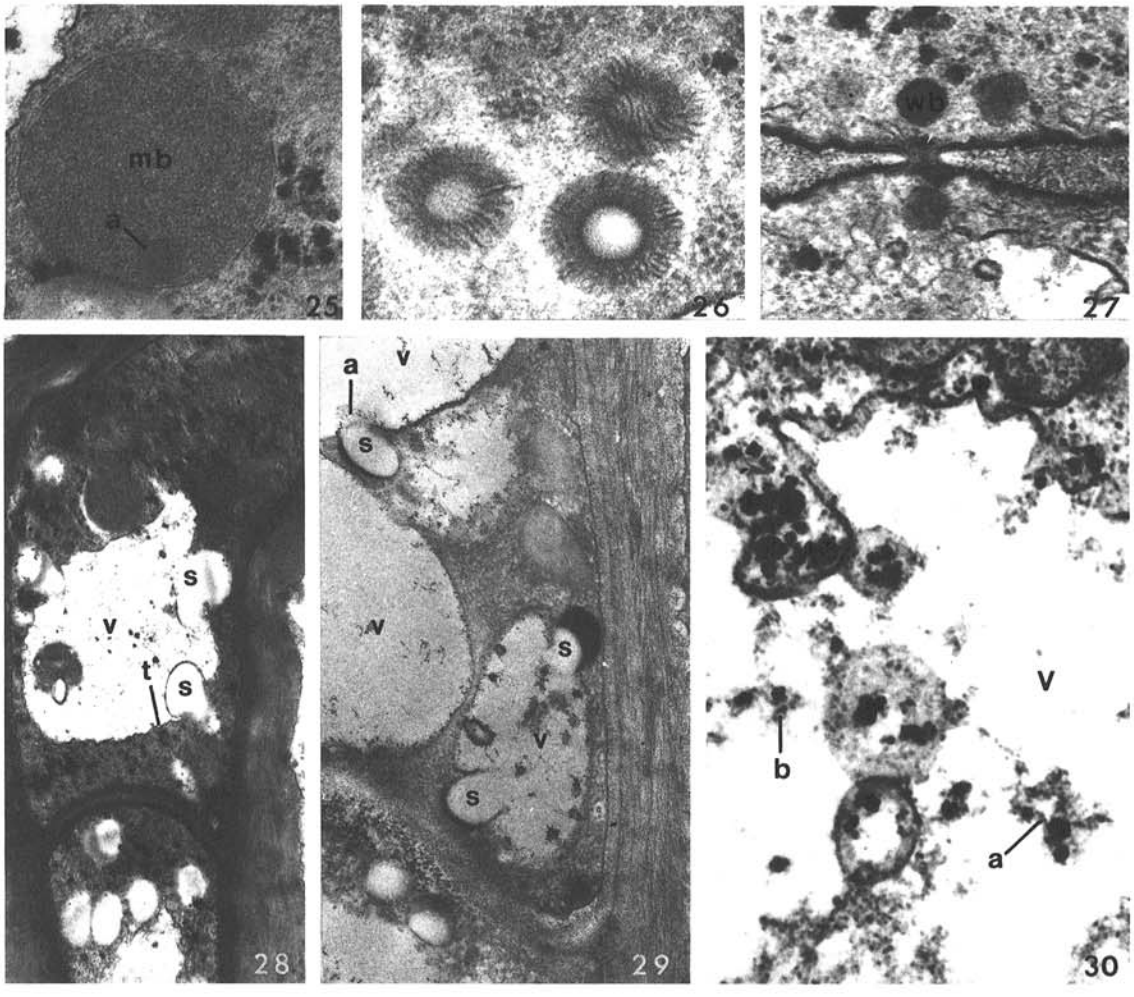


**Fig. 25-30.** Electron micrographs of sections through cells of *Elsinöe wisconsinensis*. **25)** A microbody (mb) containing a crystal (a). × 44,000. **26)** A large coated vesicle in an outer stroma cell. × 76,000. **27)** A septal pore and Woronin bodies (wb). × 50,000. **28)** Spherosomes (s) fused with the tonoplast (t) of the vacuole (v). × 15,000. **29)** Spherosomes (s) open to the vacuole (v). One spherosome at "a" appears to be discharging its contents into the vacuole (v) × 20,000. **30)** Vesicles, within the vacuole, originating from the tonoplast and containing glycogen granules. × 38,000. Note what appears to be a portion of the remaining membrane of a decomposing vesicle at "a" and the digestion of glycogen granules at "b" within the vacuole (V).

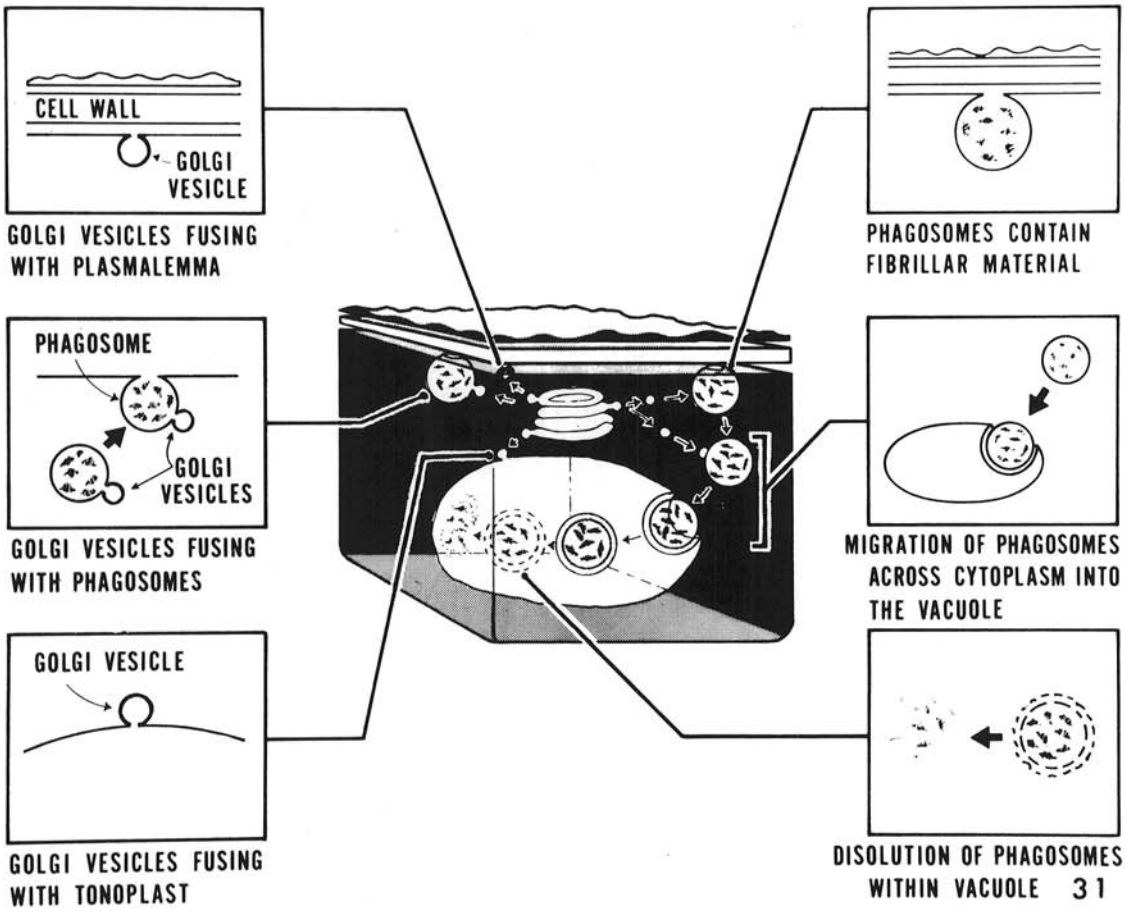


**Fig. 31.** A schematic interpretation of the interactions of various organelles and vesicles within diseased host cells of *Desmodium* illinoense infected by *Elsinöe wisconsinensis.*

granular material originate as localized invaginations of the host plasmalemma in regions where the host and pathogen are in close association. They appear to detach from the plasmalemma and pass through the cytoplasm. Golgi vesicles fuse with the phagosomes which move toward the central vacuole and become enclosed in a tonoplastic invagination. The double-membrane-bound vesicles pinch off into the central vacuole where their membranes are ultimately broken down. Golgi vesicles also interact with the plasmalemma and tonoplast in regions where the host and parasite are closely associated. The scheme illustrated is very similar to that presented by Wattiaux (12) for the lysosomal system in animal cells.

The major components of an animal lysosomal system can be found in plants. These include a Golgi apparatus (10), hydrolase-containing Golgi vesicles (2), and autophagic vacuoles (secondary lysosomes) (4). Coulomb (1) Matile (8), and Wilson (13) have indicated an interrelationship of all these components in a higher plant cell. Although conclusive evidence is not available, a lysosomal system appears to be operating in diseased *Desmodium* cells.

## LITERATURE CITED

1. COULOMB, P. 1971. Phytolysosomes dans les frondes D'Asplenum fontonum (Filicinees, Polypodioceis) isolement sur gradient dosages de quelques hydrolases et controle des culots obtenus par le microscope electronique. J. Microsc. (Paris) 11:299-318.
2. COULOMB, P., C. COULOMB, and J. COULON. 1972. Origine et fonctions des phytolysosomes dans le meristeme radiculaire de la Courge (Cucurbitae pepo L. Cucurbitacee). I. Origine des photolysosomes. Relations reticulum endoplasmique-dictyosomes-phytolysosomes. J. Microsc. (Paris) 15:236-278.
3. FITZPATRICK, R. E. 1934. The life history and parasitism of Taphrina deformans. Sci. Agric. 14:305-326.
4. GAHAN, P. B. 1968. Lysosomes. Pages 228-237 *in* J. B. Pridham, ed. Plant cell organelles. Academic Press, New York.
5. GRUEN, H. 1959. Auxins and fungi. Annu. Rev. Plant Physiol. 10:405-440.
6. MARTIN, E. M. 1940. The morphology and cytology of Taphrina deformans. Am. J. Bot. 27:743-751.
7. MASON, D. L., and M. P. BACKUS. 1969. Host-parasite relations in spot anthracnose of Desmodium. Mycologia

61:1124-1141.
8. MATILE, P. 1975. The lytic compartment of plant cells. Cell Biology Monographs, Vol. 1 Springer-Verlag, New York, Wien. 183 p.
9. MOLLENHAUER, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39:111-112.
10. NORTHCOTE, D. H. 1971. The Golgi apparatus. Endeavour 30:26-33.
11. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-213.
12. WATTIAUX, R. 1969. Biochemistry and function of lysosomes. Pages 1159-1178 *in* A. Lima-De-Faria, ed. Handbook of molecular cytology: Frontiers of biology (Monogr. Ser.). North-Holland, Amsterdam, and London.
13. WILSON, C. L. 1973. A lysosomal concept for plant pathology. Annu. Rev. Phytopathol. 11:247-272.