Host-Parasite Relationships Between *Pseudopeziza trifolii* f. sp. *medicaginis-sativae* and Alfalfa

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ABSTRACT

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Pseudopeziza trifolii f. sp. *medicaginis-sativae*, the causal agent of common leaf spot of alfalfa, produced ascomata in restricted lesions in living alfalfa leaflets. Light and electron microscopy indicated that during early disease stages, intracellular hyphae invaded the host tissues, resulting in breakdown of the protoplasts of many of the infected plant cells. In the area occupied by the developing ascoma, entire parenchyma cells were replaced by fungus tissue. Beneath the mature ascoma, spongy parenchyma cell contents were replaced by hyphae, but many of the host cell walls were not destroyed. At the lesion margins, hyphae extended into host cells that contained intact organelles. The fungus remained within host tissues until the time of ascospore dispersal. Possible reasons for the success of *Pseudopeziza* as a parasite of alfalfa include the ability of the fungus to penetrate directly into host cells, the relatively short time period between inoculation and production of fresh inoculum, and the sequestration of the fungus inside plant tissues during a major portion of the fungus' life cycle.

Additional key words: *Medicago sativa*, fungus-plant relations.

Pseudopeziza trifolii (Biv.-Bern.:Fries) Fckl. f. sp. *medicaginis-sativae* (Schmiedeknecht) Schüepp is the cause of common leaf spot of alfalfa (*Medicago sativa* L.). The fungus is frequently referred to as *P. medicaginis* (Lib.) Sacc., but Schüepp (38) noted that there is very little difference between the two species other than host. The forma specialis designation is therefore used, and *P. medicaginis* is considered a synonym of *P. trifolii. Pyrenopeziza medicaginis* Fckl., now called *Leptotrochila medicaginis* (Fckl.) Schüepp (38), is the fungus that causes yellow leaf blotch of alfalfa and should not be confused with *Pseudopeziza medicaginis*.

Common leaf spot is found worldwide and ranks among the important foliage diseases of alfalfa (11,14). Interactions between the fungus and its host have been examined with light and electron microscopy (7,16,19,32,36,38,42,43); the current study was undertaken to supplement these studies and to provide a more complete account, particularly at the ultrastructural level, of the interactions between the fungus and the host plant as the disease progresses. This work should contribute to clarifying the reasons for the success of the fungus as a parasite and should provide information about the nature of disease development and how this relates to patterns of development in other diseases.

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MATERIALS AND METHODS

Studies were done on the alfalfa cultivar Buffalo. Potted plants placed outdoors and plants artificially inoculated in growth chambers were observed to obtain information about length of time needed for disease development. Procedures for isolation and culture of *P. trifolii* from alfalfa, plant inoculations, sections of living material, paraffin sections, and electron microscopy have been described elsewhere (25). All electron microscopic observations were from field-collected specimens.

Histochemistry. Infected leaflets from field-grown plants were sectioned with a Hooker microtome. Tests for phenols were made with methods from Ling-Lee et al (20), Feder and O'Brien (9), Reeve (33), Hathway (15), and Dinkel (8). Suzuki's saturated water solution of o-dianisidine (3,3′-dimethoxybenzidine) was applied for 1–16 hr in the dark to stain for quinones (26).

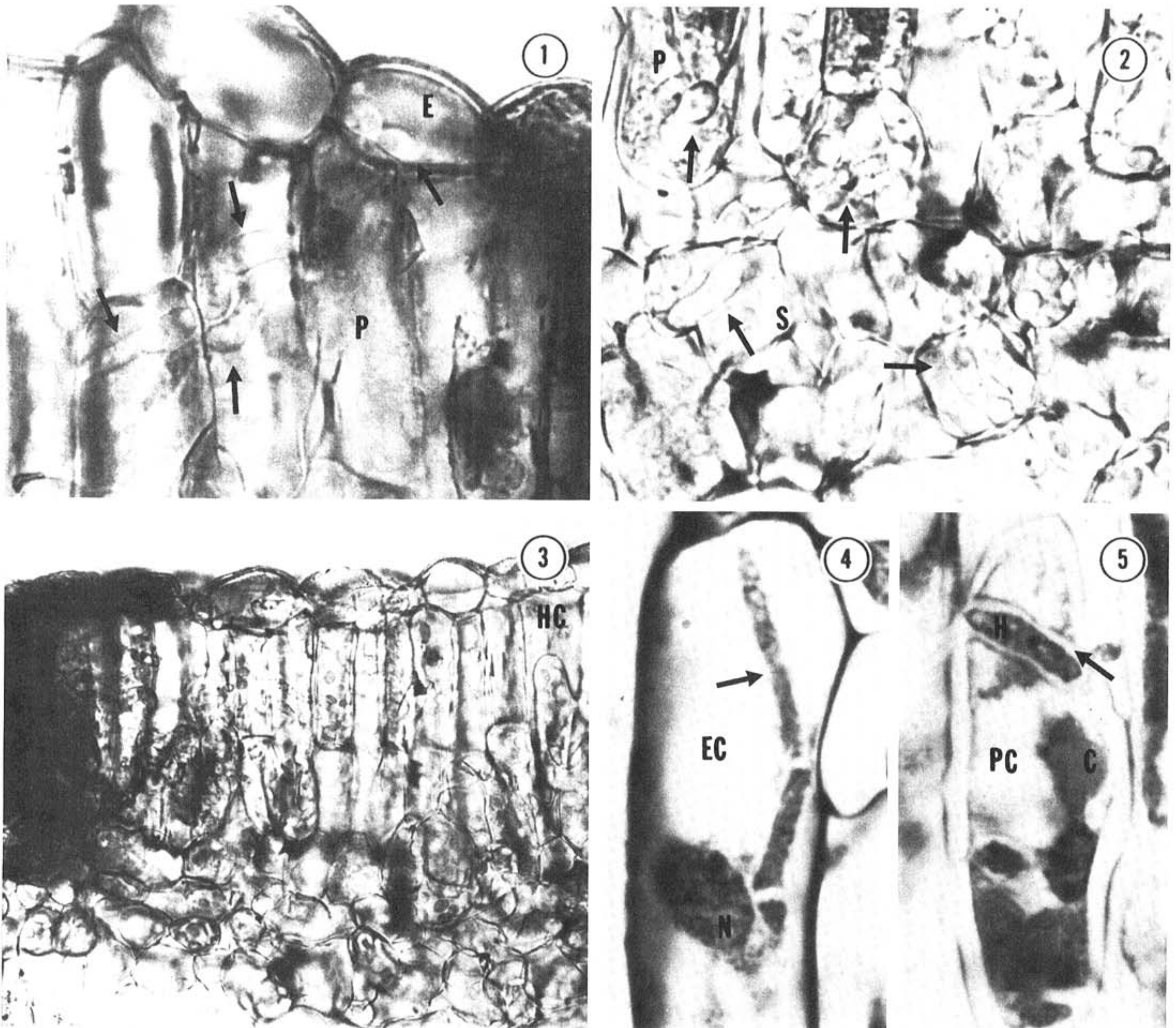
Development of ascomata in excised and dead leaves. In the first experiment, two groups of 50 leaves each were selected from field-grown plants. Group 1 consisted of leaves with spots, but no ascomata sufficiently developed to raise the host epidermis. Group 2 consisted of leaves with mature ascomata and recognizable developing ascomata (ascomata that were raising the host epidermis). Each set of leaves was randomly divided into five subgroups, and each subgroup was placed into a packet made of wire mesh. On 23 June 1982, the packets were placed aboveground among alfalfa plants in the field. Three days later, and thereafter at weekly intervals until 24 July, two leaves were collected from each packet, examined for ascoma development, wetted, and placed on

moist filter paper in petri dishes. Ascospores were allowed to discharge up onto dry cover slips. Group 1 leaves were placed on moist filter paper near a window for an additional 3–5 days to see if ascomata would develop.

In the second experiment, infected leaves from the field were divided into four groups, placed into wire packets, and placed among alfalfa plants in October 1982. Group 1 leaves had spots that showed no external evidence of ascoma production. Group 2 leaves contained immature ascomata (defined here as developing ascomata that had not ruptured the host epidermis) as well as younger spots. Group 3 consisted of leaves with infections ranging from small lesions to fully erumpent ascomata. Group 4 was composed of leaves that were yellowing and falling off of plants. Most group 4 leaves had some externally identifiable ascomata in various stages of development.

One wire packet from each group contained a single layer of leaves; one to two additional packets containing four to five leaf layers were made for groups 1–3. Two weeks later, and once a month thereafter until April 1983, leaves were collected from the packets. The leaves were wetted, placed on moist filter paper in petri dishes, examined for the presence of mature ascomata, and inverted over 1.5% water agar so that ascospore discharge could take place overnight. The agar was then checked for ascospores, and the leaves examined for turgid ascomata.

The third experiment was done in the lab to check for comparative development of ascomata in living and dead leaves. Thirty-four infected leaves with young spots about 1 mm or less in diameter that showed no external evidence of ascoma development were selected from field-grown plants. Seventeen leaves were laid on moist filter paper in large petri dishes, and the petri dishes were placed next to a window. The dishes were kept at an angle so that the petioles of the leaves were in water while the leaflets were on the moist filter paper. The other 17 leaves were laid on newspapers between blotters and allowed to air dry. Five days later these leaves



Figs. 1–5. *Pseudopeziza trifolii-Medicago sativa*. **1–3,** Sections of living leaflets showing spread of the hyphae through the host. **4 and 5,** Paraffin sections of infected cells. **1,** Hyphae (arrows) growing from the upper epidermis (E) to the palisade parenchyma (P). ×1,000. **2,** Hyphae (arrows) growing from the palisade parenchyma (P) to the spongy parenchyma (S). ×1,000. **3,** Gradation in host-parasite interaction from the center of the lesion, which contains hyphae and brown substances and appears dark in this micrograph, to healthy cells (HC) in the leaflet. ×420. **4,** Infected, uncollapsed epidermal cell (EC) with a recognizable nucleus (N). Arrow indicates hypha. ×2,000. **5,** Infected palisade parenchyma cell (PC) with a nucleus and somewhat misshapen chloroplasts (C). Host cell plasmolysis emphasizes the zone (marked with arrow) between the hypha (H) and the host protoplast. ×2,000.

were removed from the papers, wetted, and treated like the first set of leaves. All leaves were checked periodically over a month's time for ascoma development.

RESULTS

Infection. On naturally infected plants growing outdoors, the time required for spots recognizable as *Pseudopeziza* infections to develop varied from less than a week to more than a month after a leaf opened. On artificially inoculated plants, spots appeared in 5–13 days, and young ascomata containing some ascospores formed within 14 days after inoculation.

Histology and ultrastructure of infection. Infection commonly proceeded from the upper epidermis (Fig. 1) and will therefore be described as occurring in this manner. However, infections also started from the lower epidermis. Ascospores on the leaf surface were frequently in pairs, surrounded by a common matrix (Fig. 7). The ascospore germ tube penetrated directly through the cuticle and epidermal cell wall into the host epidermal cell. Epidermal cells surrounding the initial point of infection were invaded first; the intracellular mycelium eventually grew outward and downward to the parenchyma and lower epidermis, with the hyphae passing directly from host cell to host cell (Figs. 1–3).

Epidermal cells in the center of the lesion were infected and collapsed; most of these contained brownish substances. The perimeter of the collapsed area was usually composed of infected but uncollapsed epidermal cells (Figs. 1 and 3), some of which had recognizable nuclei (Fig. 4).

In the parenchyma layers, centrally located cells also generally contained brown substances (Fig. 3). The brown substances in the epidermis tended to extend beyond those in the palisade layer (Fig. 3). Sometimes brownish parenchyma cells that still appeared to have chloroplasts were present. The lesion also contained infected cells that did not have brown substances, but seemed to have disrupted contents. There were generally other infected cells, especially at the edge of the area with brown substances, that appeared healthier, with nuclei and chloroplasts distinguishable in the light microscope (Figs. 3 and 5); sometimes these organelles appeared normal, although in other cases the chloroplasts seemed somewhat misshapen (Fig. 5).

The diameter of the infected area was often broader in the upper leaf layers than it was in the spongy parenchyma. However, hyphae moved downward between layers at more than one point (Fig. 2); lateral spread in a cell layer did not depend solely on outward hyphal growth from a central point within that layer. As mentioned, lower epidermal cells were eventually infected, and collapsed sometime later. As in the upper epidermis, the collapsed plant cells retained their walls.

Throughout development of the lesion, hyphae generally extended beyond the central area into host cells that appeared healthy in the light microscope (Fig. 3). Some spots had small, yellowish halos; hyphae were observed extending across the area of the halos into parenchyma cells with green chloroplasts.

Ascomatal initials formed in infected parenchyma cells in young lesions. Some of the parenchyma cells, especially in the palisade, had hyphae massed in them; the internal host contents were disorganized, and the cells became distorted. This was followed by degeneration and tearing of many of the host cell walls (Fig. 12). Remnants of the brown substances were still present in the palisade cells at this time. Sometimes palisade cells near this area contained much mycelium, and still had nuclei and even chloroplasts, although the organelles did not generally appear healthy. Hyphae tended to be less dense in the spongy parenchyma, and some of these host cells still had organelles (Fig. 13). Some intercellular hyphae formed (Fig. 13).

As the ascoma developed and the lesion increased in diameter, host cells in the ascomatal area were almost completely filled by hyphae, although the hyphae were interspersed with occasional host protoplast remnants. Large gaps formed in the walls, and hyphae grew both in the area once occupied by host cells and in the former intercellular spaces. Infected spongy parenchyma cells directly under the area of heavy infection often retained some internal structure for a while.

A hymenium of asci and paraphyses was produced, replacing the

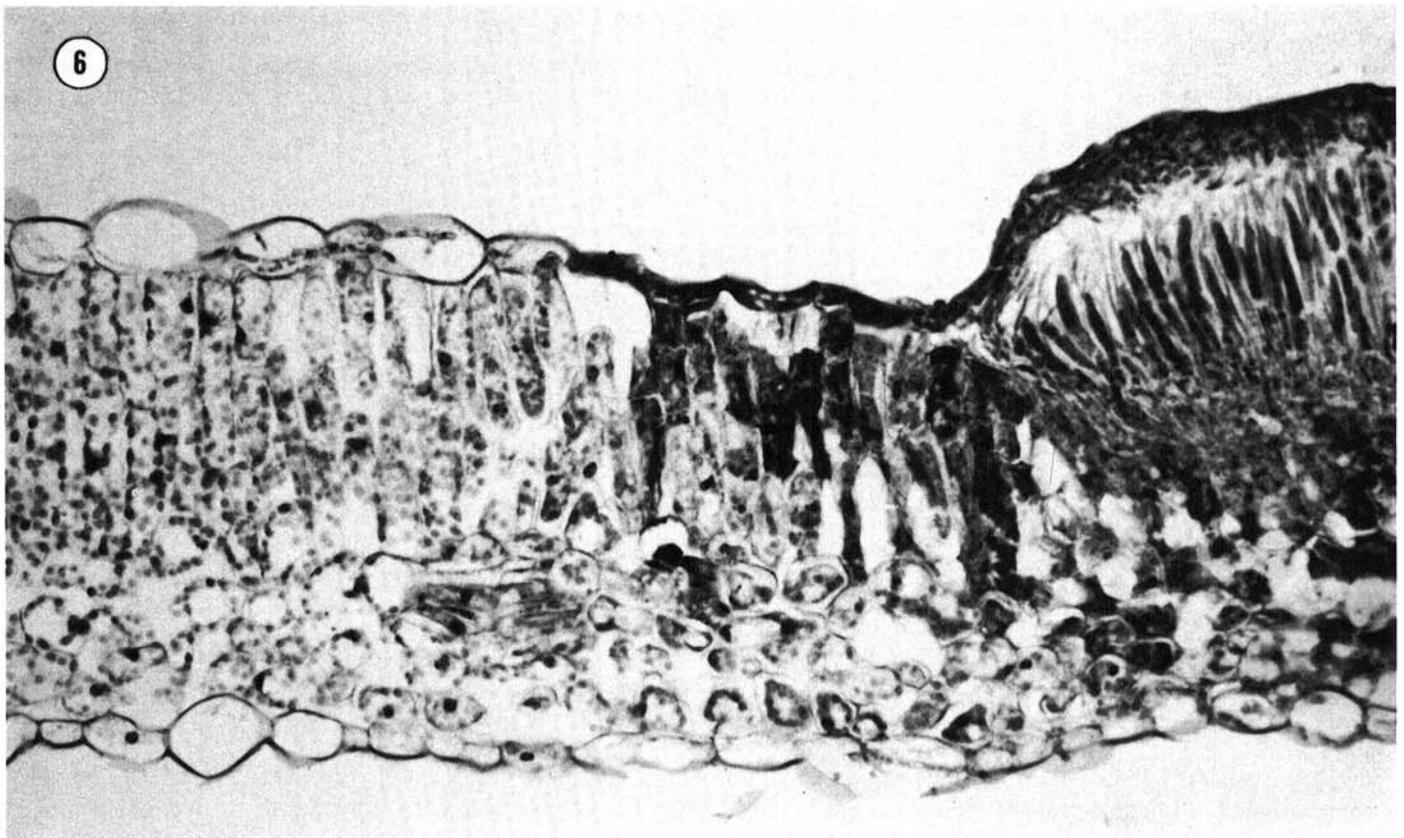


Fig. 6. *Pseudopeziza trifolii-Medicago sativa.* Paraffin section showing gradation in host cell response from the lesion center, where the ascoma is located, to healthy leaflet cells near the lesion margin. ×400.

palisade parenchyma cells. The palisade cells within the ascomatal periphery frequently had basal walls; in this area, at least the uppermost walls of the palisade cells were broken down. Remnants of disorganized host protoplasts, cell walls, and brown substances were present among the developing hymenial and subhymenial elements. These remnants disappeared as the hymenium matured. The hymenium raised the host epidermis above it as it grew, and some remnants of palisade cells and brown substances were often attached to the epidermis.

Portions of palisade cells and brown substances, or entire and often brown palisade cells, generally remained under the young hymenium. Host parenchyma cells beneath the hymenium had the protoplasts replaced by fungal hyphae and often contained brown substances; walls of some cells were partially disorganized, but many cells retained the walls. Some of these host cells appeared smaller than the healthy parenchyma cells, perhaps because of some collapse. Heavily infected spongy parenchyma cells at times intergraded with the developing hymenium and subhymenium above them, with partial destruction of the upper spongy parenchyma cells often occurring. Some lower spongy parenchyma cells still had nuclei; chloroplasts, when present, usually did not appear healthy. The lower epidermal cells in this area were generally collapsed and frequently showed a brownish tinge.

As the ascoma matured, it ruptured the infected epidermis, which remained attached around the ascoma edge. The ascospores were exposed to the external environment for dispersal. Ascomata also formed in the lower epidermal region, sometimes opposite ascomata in the upper epidermal region of a leaf spot. Brown substances surrounded many ascomata (Fig. 6). Host cells were replaced by fungus tissue in the basal part of the ascoma; sometimes a few wall remnants opened into this region, or traces of brown substances remained. The bottom portions of some palisade cells persisted under the ascomata.

From the ascoma to the lower epidermis was an area, generally of spongy parenchyma, where fungal hyphae and brown substances had replaced the contents of many host cells (Fig. 14). Host walls, or portions of them, were present. In fresh sections, the brown substances did not always appear as dark in the lower parenchyma of the leaf, and infected spongy parenchyma cells with chloroplasts or nuclei were occasionally found above the lower epidermis. The lower epidermis was infected, generally contained brown substances, and had collapsed cells. In cross sections, many leaves appeared slightly smaller where the ascoma was located.

At the ascoma edge, epidermal cells contained brown substances and were collapsed. Uncollapsed brownish epidermal cells were often present next to the collapsed cells, and beyond these were infected cells without brown substances. Palisade cells at the ascoma perimeter were frequently heavily infected and brownish (Fig. 6). The brown substances sometimes seemed a little lighter in the palisade parenchyma than in the epidermis. Host cells around the ascoma at times appeared disrupted; not only were the host contents gone, but parts of walls were missing.

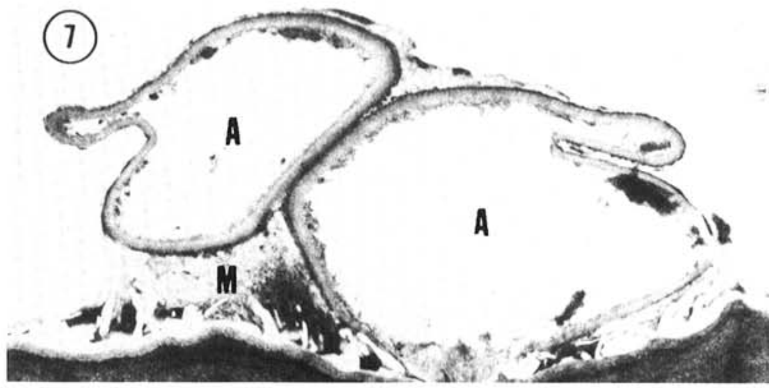
In the parenchyma as well as in the epidermis, hyphae extended out from the ascomatal area through the less affected cells to healthy looking host cells (Fig. 6). Sometimes the chloroplasts in these infected cells were a paler green than the chloroplasts in uninfected cells.

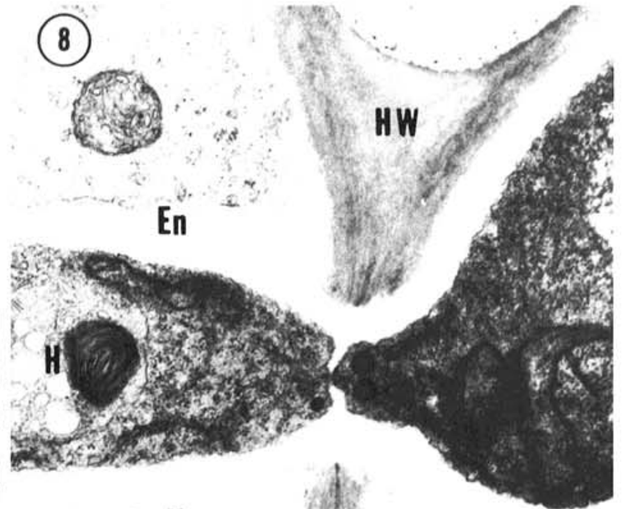
Electron micrographs demonstrated that the hyphae spread throughout the tissue by constricting, passing through the walls, and enlarging in diameter again once a new cell was reached (Fig. 8). When a hypha entered a cell, the host plasmalemma was invaginated around the hypha, remaining continuous with the plasmalemma in the rest of the host cell (Figs. 9,10,16). When a hypha exited a cell, the invaginated host plasmalemma fused with the plasmalemma in the area where the hypha grew through the wall and left the cell (Fig. 9). An encapsulation separated the fungus wall and the invaginated host plasmalemma (Figs. 8–11,13). The encapsulations were electron-lucent (Figs. 8–11,13). The width of an encapsulation was variable, even around a single hypha (Figs. 8–11,13). Host endoplasmic reticulum was occasionally observed to be arranged parallel to the host plasmalemma and encapsulation. As the host cell contents were disrupted, the plasmalemma started to break up, and in highly disorganized cells was no longer present (Fig. 23). Electron-opaque substances in host cells corresponded in position to the brown substances seen in fresh sections. When observed with electron microscopy, these substances varied from smooth to granular in appearance (Figs. 11,12,14,23).

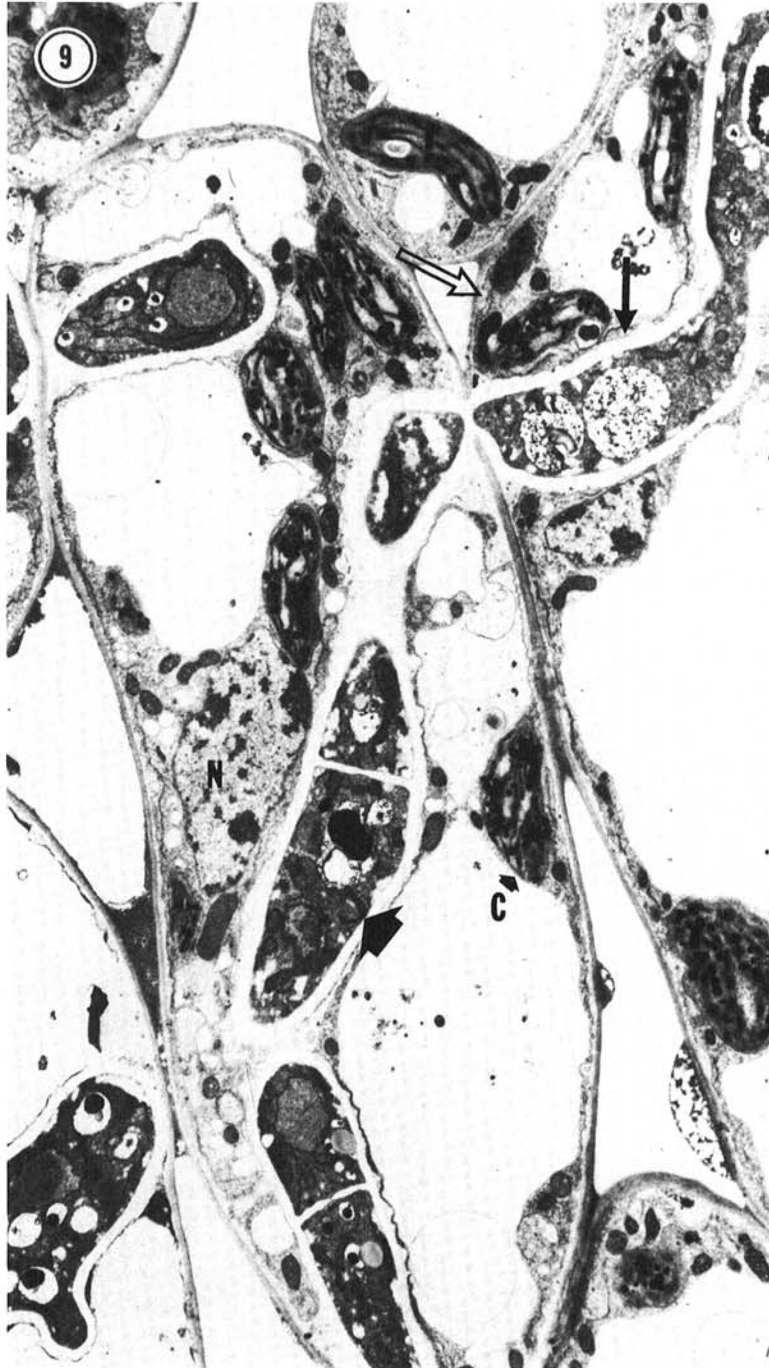
The ultrastructure of the infected, collapsed host epidermal cells is demonstrated in Figure 11. Electron-opaque substances were present around the hyphae, and the host cell wall was still present. Many ultrastructural changes occurred in infected host parenchyma cells undergoing disorganization in younger lesions and near the margins of older lesions. The disruption of palisade cells included loss of the host tonoplast and central vacuole, disorganization of organelles, and loss of the plasmalemma. Chloroplast and nuclear envelopes and chloroplast lamellae were disrupted and disorganized. Plastoglobuli generally increased in number or size (Figs. 21,22). In some cells, chloroplasts became somewhat rounder in appearance (Fig. 22). For a while after fragmentation of the nuclear envelope, the nucleoplasm was distinguishable (Fig. 21). Clumping of organelles frequently occurred (Fig. 11), and often the host cell breakdown resulted in formation of electron-opaque areas with little or no discernible structure (Fig. 23). Internal cell contents persisted, at least for a short time, in many parenchyma cells after infection. Hyphae grew across cells that still contained vacuoles, chloroplasts, nuclei, mitochondria, ribosomes, and other organelles (Fig. 9).

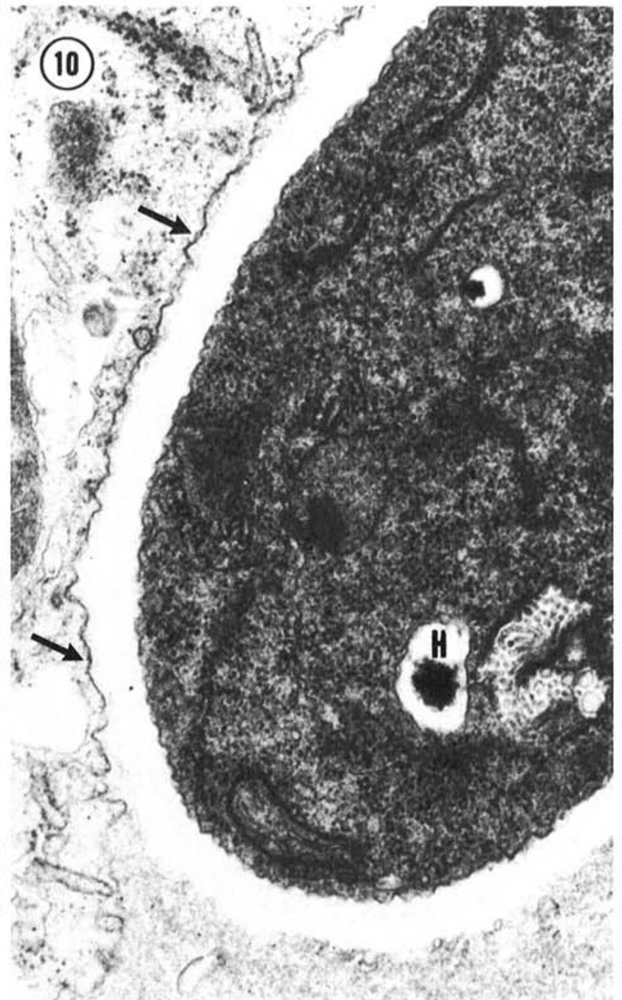
After ascoma development, a gradation in host-parasite interactions from the ascoma region to the periphery of the lesion could still be seen in the electron microscope as well as in the light microscope. Heavily infected cells were under the ascoma. Parenchyma cells further from the lesion center were often infected and unhealthy looking. Many of the nuclei and chloroplasts in these plant cells were senescing. In some specimens, intercellular hyphae were found in this area. At the lesion margin, the effect on the host cells was quite different. Hyphae extended into cells that still contained intact organelles. Comparison between infected parenchyma cells in this region and uninfected parenchyma cells indicated that infected parenchyma cells at the border of the spot appeared similar to uninfected cells, although modifications could occur. Uninfected parenchyma cells had organelles arranged in a peripheral band of cytoplasm around a central vacuole (Fig. 15). In some of the infected cells near the lesion margin, there seemed to be rearrangement of organelles, which often were farther from the walls than usual (Fig. 16). This may have been a result of vacuolar shrinkage. Endoplasmic reticulum and ribosomes were present in both types of cells, and the plasmalemma and tonoplast were intact. Chloroplasts in both infected and uninfected cells had plastoglobuli, well-organized thylakoid structure with granal stacks, and often, starch (Figs. 15–18). Envelopes of chloroplasts in infected cells were not always as sharply delimited as the chloroplast envelopes in uninfected cells. Mitochondrial structure appeared unaltered (Figs. 17–20). Nuclei had well-developed envelopes, electron-opaque condensed chromatin, and interchromatin regions of similar electron opacity in infected and uninfected cells (Figs. 15,16,19,20). These infected cells thus appeared quite different from the previously described parenchyma cells undergoing disorganization in younger lesions or near the margins of older lesions.

The substances that appeared brownish in fresh sections and electron-opaque with electron microscopy were tested with various stains to determine their nature. Most of the histochemical stains used on these substances produced either negative or inconclusive results. Much of the brown area did stain positively in o-dianisidine (3,3′-dimethoxybenzidine), indicating that quinones might have been present. However, in paraffin sections stained with hematoxylin, both a greenish-yellow and a dark purplish-blue color appeared in areas corresponding to the brown substances in fresh material. Infected epidermal cell contents were usually greenish-yellow, whereas mesophyll cells stained either color. This differential staining indicated that more than one substance was present.

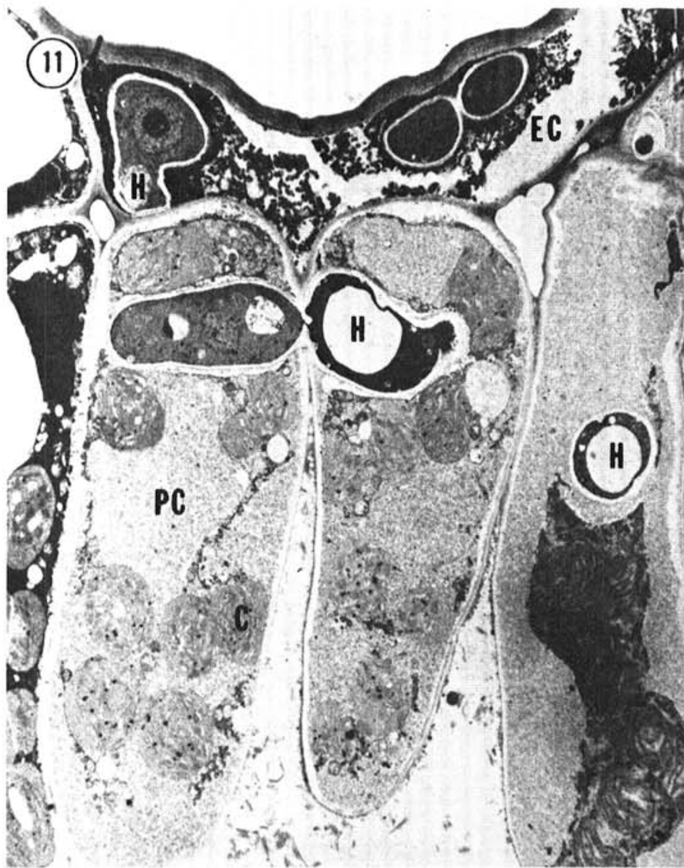


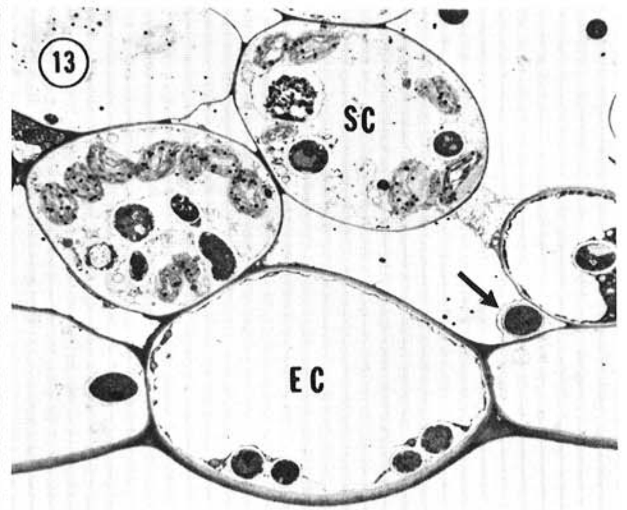


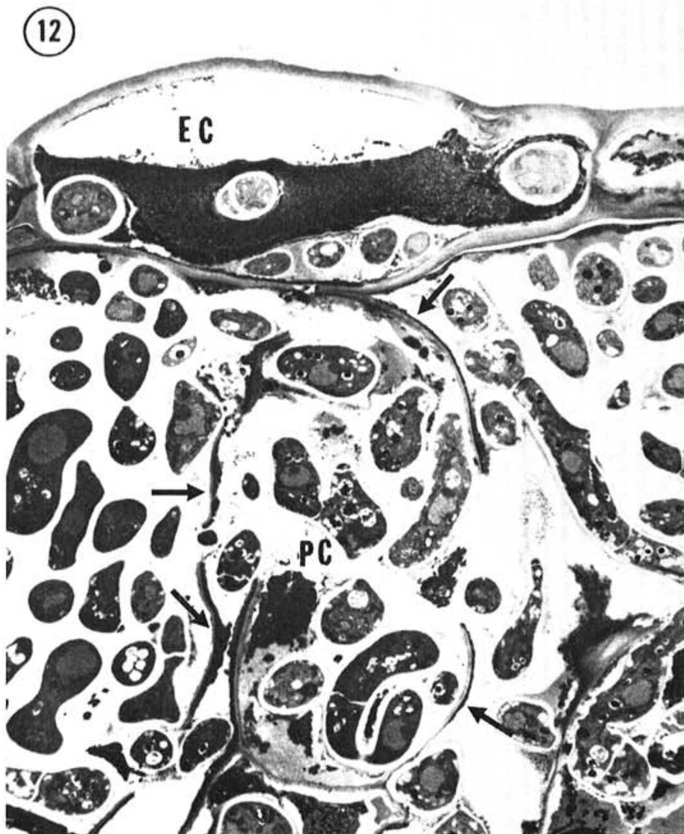


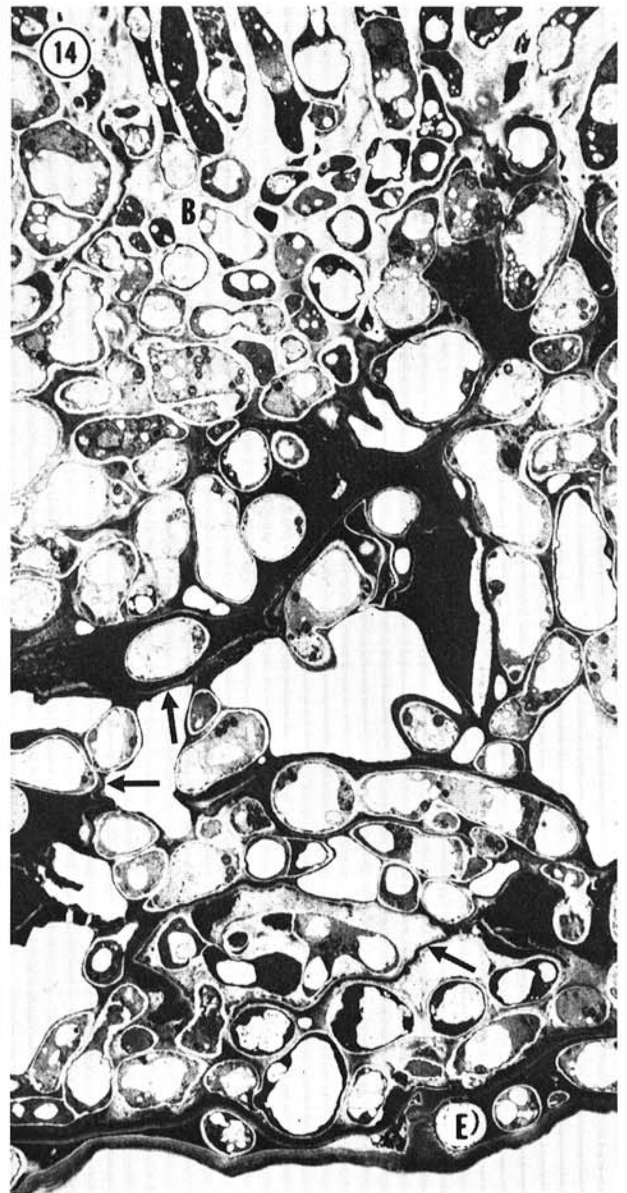


Figs. 7–10. *Pseudopeziza trifolii-Medicago sativa.* Transmission electron microscopy of ascospores and of hyphae in host cells. **7,** Paired ascospores (A) on the leaflet surface. Both ascospores are enveloped in a matrix (M). ×12,800. **8,** Hypha (H) growing between host cells (Ho). The hypha constricts as it passes through the host wall (HW). An encapsulation (En) is present. The hypha has a septum that contains a pore. ×21,800. **9,** Hypha (wide arrow) in a parenchyma cell. The host cell retains its structure. The chloroplasts (C), nucleus (N), and other organelles are still present. The uninvaginated host plasmalemma (light arrow) is fused with the invaginated host plasmalemma (dark arrow). ×5,600. **10,** Irregular, invaginated host plasmalemma (arrows) separated from the hypha (H) by an encapsulation. ×37,500.

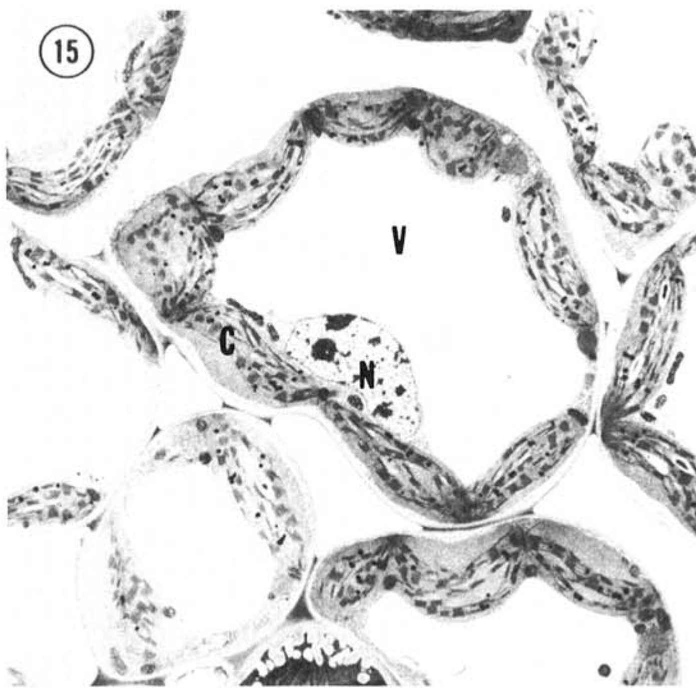


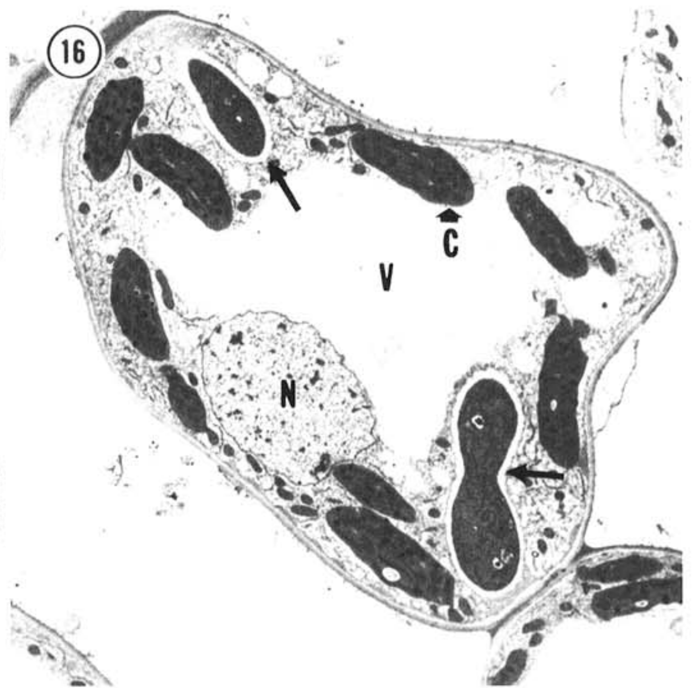


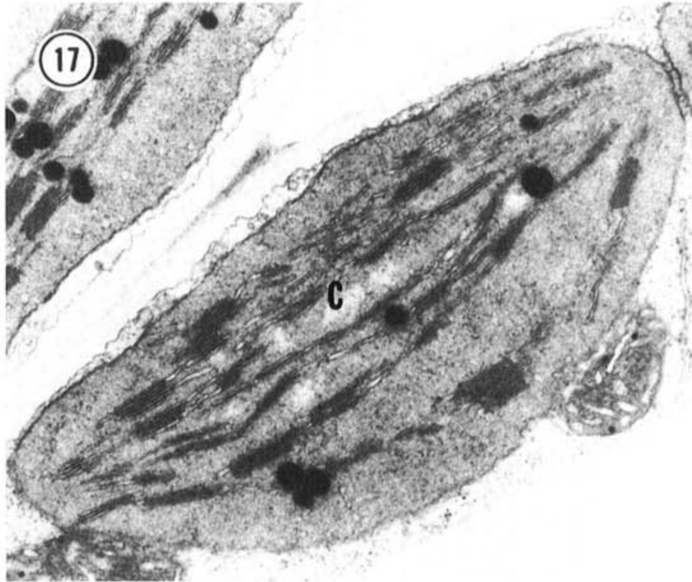


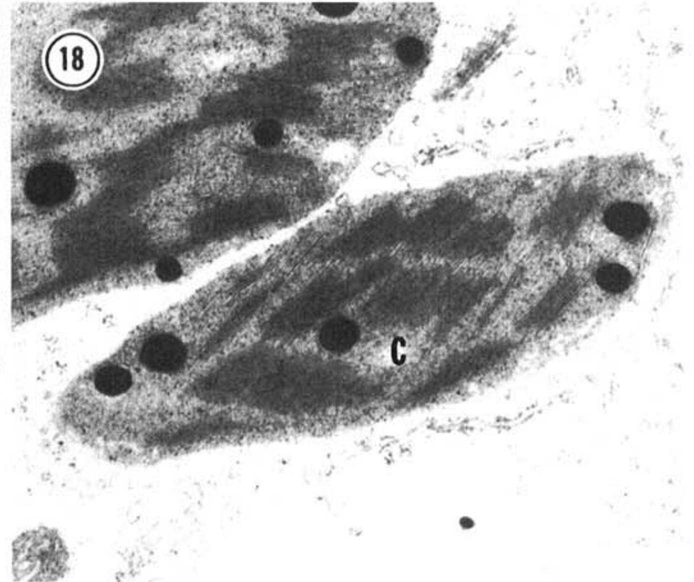


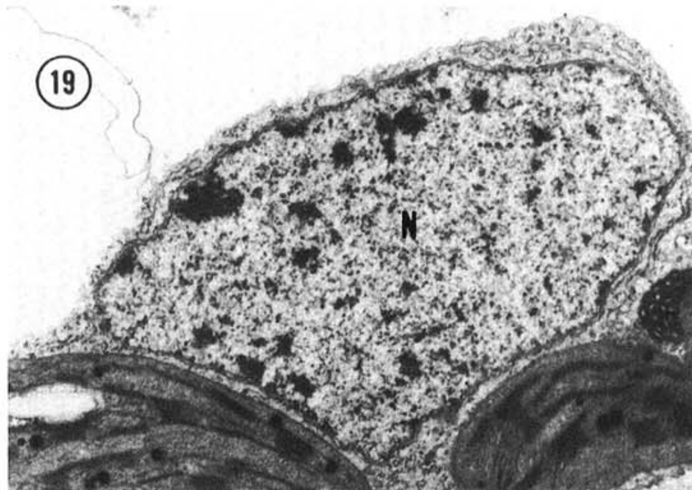
Figs. 11-14. *Pseudopeziza trifolii-Medicago sativa.* Transmission electron microscopy of host cell breakdown. **11,** Collapsed epidermal cell (EC) overlying degenerating palisade cells (PC). Hyphae (H) are present, and chloroplasts (C) can still be discerned. ×3,400. **12,** Degenerated palisade cells (PC) with internal contents mostly replaced by hyphae. Walls (arrows) of host palisade parenchyma cells are being disrupted. Walls of host epidermal cells (EC) are intact. ×2,400. **13,** Spongy parenchyma cells (SC) and epidermal cells (EC) underlying the area where the palisade parenchyma cells are being disrupted. The host cell protoplasts in this area have not been replaced by fungal structures. An intercellular hypha (arrow) is present in this area. ×1,900. **14,** Leaflet cross section from the base of the fungal hymenium (B) to the lower host epidermis (E). The hymenium has replaced host tissue, while much of the parenchyma under the ascoma still retains the host cell walls. Some of the host walls are indicated with arrows. ×2,200.

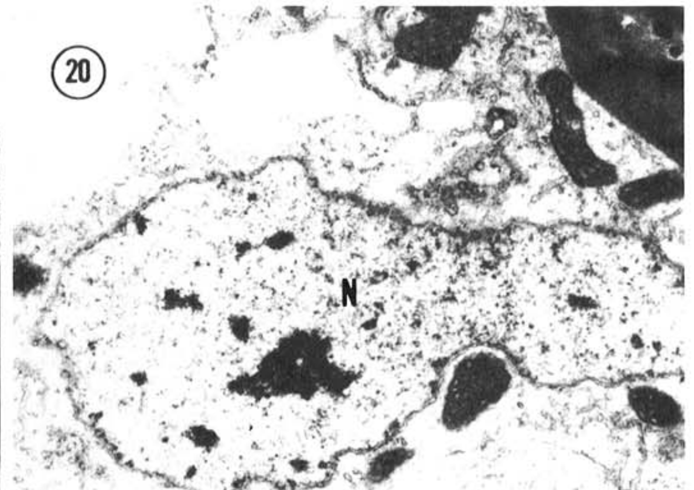










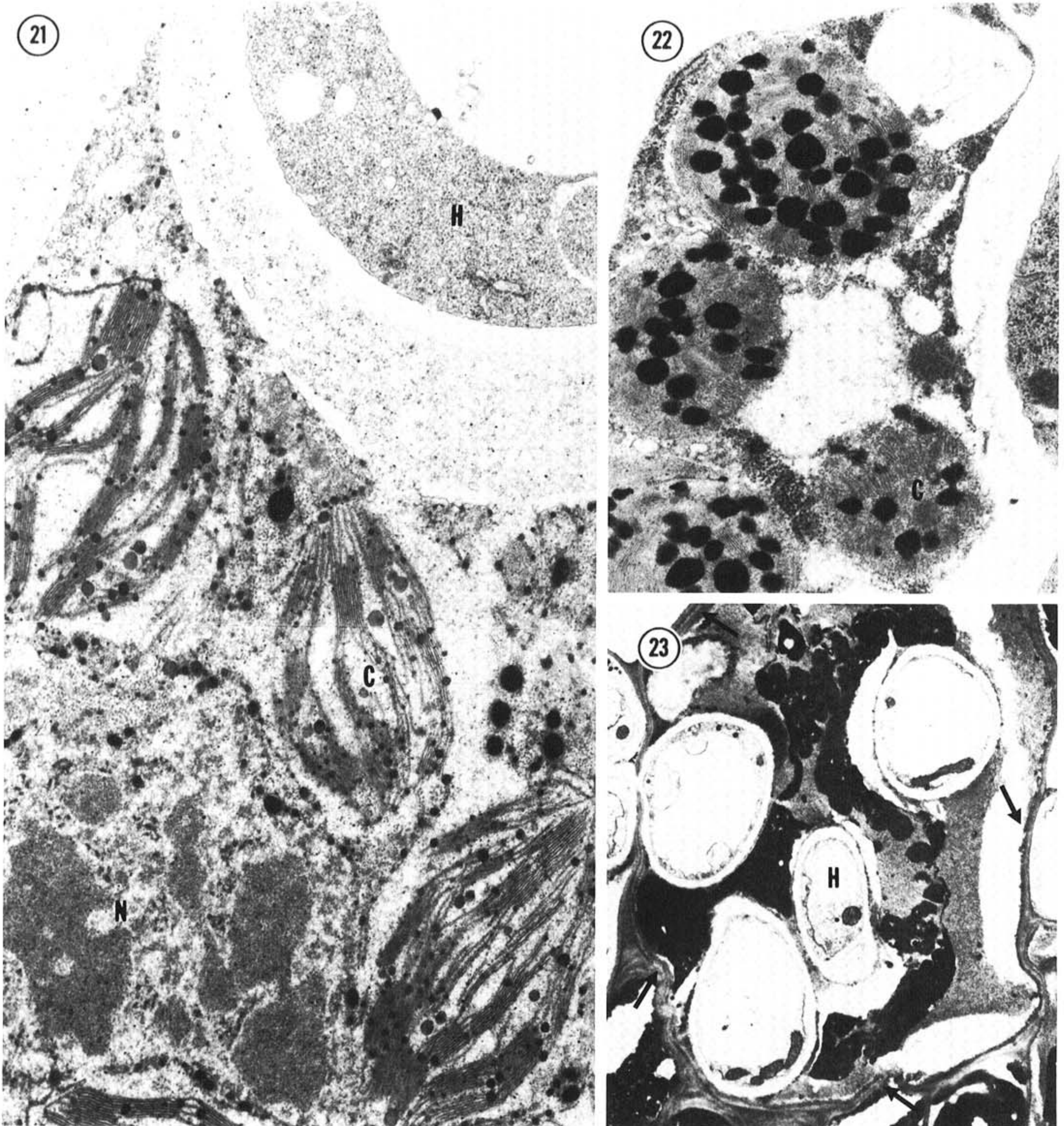


Figs. 15–20. *Pseudopeziza trifolii-Medicago sativa.* Transmission electron microscopic comparison between organelles in healthy cells and organelles of infected cells at the margins of mature lesions. **15,** Healthy parenchyma cell with a large central vacuole (V), a nucleus (N), and chloroplasts (C). ×3,400. **16,** Infected parenchyma cell. The hyphae are marked with arrows. The host nucleus (N), vacuole (V), and chloroplasts (C) are still present, as are the other cellular organelles, but the chloroplasts generally appear to be farther from the host cell wall. ×3,400. **17,** Chloroplasts (C) from a healthy cell. ×31,200. **18,** Chloroplasts (C) from an infected cell. The chloroplast envelopes are not as distinct as in the uninfected cell. ×31,200. **19,** Nucleus (N) from a healthy cell. ×13,500. **20,** Nucleus (N) from an infected cell. ×13,500.

Production of inoculum on detached leaves. Infected leaves placed outdoors in packets in the summer seemed able to provide at least some inoculum source. Leaves that initially had no externally identifiable ascomata did not develop mature ascomata, either in field packets, or later on moist filter paper in the lab. Leaves that initially contained mature ascomata, as well as younger stages, continued to discharge ascospores for nearly a month. The last leaves were collected one month after placement outdoors in wire packets, and although some ascomata were present, no *Pseudopeziza* ascospore discharge was observed.

The results obtained over winter were somewhat different. Many of the yellowing leaves had ascomata when collected; however, *Pseudopeziza* ascospores were collected from these leaves only in October, about 2 wk after the packets were placed in the field. Greener leaves that initially had mature ascomata usually had mature ascomata up through December; after that, mature ascomata were only found in April, on layered leaves. *Pseudopeziza* ascospores were collected in October from both types of packets and in November from layered leaves.

Leaves containing immature ascomata produced mature



Figs. 21–23. *Pseudopeziza trifolii-Medicago sativa.* Transmission electron microscopy of host cell degeneration. **21,** Breakdown of host cell organelles. A hypha (H) is present. The chloroplasts (C) and nucleus (N) are becoming disorganized. ×24,000. **22,** Degenerating chloroplasts (C). The envelopes are disorganized, and in this specimen, plastoglobuli are numerous. ×25,000. **23,** An infected lower epidermal cell filled with hyphae (H) and with electron-opaque areas that correspond to the brown substances seen with light microscopy. The host cell wall is indicated with arrows. ×5,900.

ascomata in October and November, with some ascomata appearing in the months of December, January, and April. Leaves arranged in one layer produced ascomata for a longer time than layered leaves. However, only a few ascospores were collected; this occurred in December, from layered leaves.

Leaves that initially had spots but no obvious raising of the host epidermis also produced a few ascomata, in November and December on layered leaves, and in October and April on leaves that were in one layer. A few ascospores were collected in October and December from leaves in one layer, but no ascomata were visible in those particular leaves, perhaps because the ascomata were obscured by contaminating fungi.

In glass dishes, ascomata formation was significantly different between leaves that were kept alive and leaves that were air-dried before placement in dishes. Ascomata started forming on the living leaves within 4 days. The dead leaves never produced ascomata.

DISCUSSION

Common leaf spot of alfalfa bears similarities to two rather diverse categories of diseases. Macroscopic observation of the disease indicates that it may be a typical necrotic leaf spot resulting in discoloration and necrosis of host tissues in and around the lesion. Alternatively, the disease resembles local lesion diseases that are caused by biotrophs such as rust fungi, in that the ascomata appear as pustular structures under the host epidermis, and the mycelium extends beyond the narrow necrotic zone into surrounding living tissue.

Initial infection and intracellular growth do not distinguish *P. t.* f. sp. *medicaginis-sativae* from pathogens causing either type of disease. The germ tube penetrates directly through the host cuticle and wall, or through stomata, without production of an appressorium (16,27,31,32,42,43). The ascospores themselves eliminate the need for an appressorium. Many of the ascospores are discharged in pairs, and as observed by Morgan and Parbery (27) with scanning electron microscopy, each spore pair is held together by an envelope that may adhere to the cuticle of the host. Morgan and Parbery observed that the cuticular wax in the area near the envelope seemed partly dissolved, and the envelope remained intact after penetration of the leaf by the fungus. A matrix around paired ascospores was also revealed with transmission electron microscopy (Fig. 7).

After penetration, the intracellular hyphae grow from host cell to host cell by constricting as they pass through the walls, and then expanding again on reaching the neighboring cell. Bracker and Littlefield (4) stated that when one living cell is invaded by another, the invading cell has a membrane around it that is often invaginated or modified host plasmalemma. Gray et al (13), for example, described an intracellular infection structure of *Cronartium quercuum* f. sp. *fusiforme* on *Pinus taeda* that invaginated the host plasmalemma, which then fused with uninvaginated host plasmalemma at the site where the fungus exited the host cell. A similar arrangement of the plasmalemma occurs in *Pseudopeziza*.

An encapsulation separates the walls of intracellular *Pseudopeziza* hyphae from the invaginated host plasmalemma. This probably corresponds to the "extracellular amorphous substance" mentioned by Šrobárová (43) as surrounding the intracellular hyphae. Substances surrounding intracellular infection structures are a common phenomenon; sheaths, encapsulations, and extrahaustorial matrices of various origins have been found associated with fungi that cause a number of diseases, including rusts, powdery mildews, smuts, and false smut (3,4,6,10,12,13,18,22,24,30). The encapsulation surrounding hyphae of *Pseudopeziza* in alfalfa is generally electron-lucent, but some encapsulations are darker than others.

Although the parasite is usually considered to grow intracellularly, electron micrographs demonstrate that some intercellular hyphae may form. Šrobárová (42) noted intercellular growth in the parenchyma as well, and Jones (17) observed that early fungus growth is strictly intracellular, but as infection proceeds the fungus may enter intercellular spaces.

After invasion, there is evidence indicating that the organism is causing a necrotic leaf spot, and not establishing a compatible relationship with the host. An indication that the interaction is incompatible is the presence of brown substances in the host tissue. The differential staining of the brown substances in alfalfa, and the variable electron-opacity and granularity of the corresponding substances seen with EM, indicate that more than one substance is present. Some of the brown substances may be disorganized host cell contents; however, quinone formation apparently accounts for at least some of the brown color. Quinones may be produced in host-parasite interactions when phenolic substances are oxidized, and the oxidation products may be inhibitory to the fungus (26).

The breakdown undergone by many host cells after infection may also be an indication of incompatibility between this fungus and its host. Aist (1) and Akai et al (2) pointed out that degenerative effects are diverse, and that the nature of the process depends on the system under study. Ultrastructural observations of the disorganization and death of *Pseudopeziza*-infected alfalfa cells indicate that some of the degenerative processes described in other infected plant cells are involved, including disruption of the plasmalemma, tonoplast, and nuclear and chloroplast envelopes, increase in the size or number of plastoglobuli, chloroplast disorganization, destruction of other membrane-bound organelles, and coalescence of cytoplasm into an electron-opaque mass (1,5,21,23,29). Šrobárová (43) also mentioned breakdown of chloroplasts and nuclei in infected host cells, followed by destruction of entire cells. This host cell breakdown may be a means of providing nutrients for the fungus; it is also possible that the host cell necrosis, combined with the previously mentioned quinone production, could be a resistance response of the host. Schmiedeknecht (34,35), categorizing infection types of *Medicago* spp. to *Pseudopeziza*, saw responses ranging from no visible reaction, to formation of brown to black spots combined with ascoma production and defoliation. For example, infection of *Medicago sativa* by *P. t.* f. sp. *medicaginis-sativae* induced a weak host reaction, eventually allowing ascoma development. Infection by *P. t.* f. sp. *medicaginis-lupulinae* resulted in a hypersensitive response in the palisade parenchyma where only one or two cells were infected. However, when *Medicago lupulina* was infected with *P. t.* f. sp. *medicaginis-lupulinae*, a pronounced defense reaction occurred, but infection continued and ascomata developed. Therefore, host defense reactions may be a part of an interaction even if they are not effective in stopping invasion by the pathogen. If the host cell degeneration is a resistance response of the plant or a process that provides the parasite with most or all of its nutrients, then the two organisms are probably incompatible.

However, the necrotic response of alfalfa cells to this pathogen varies within a leaflet. In the epidermis, the necrosis is often accompanied by collapse of the host cell, a response that is not as common or pronounced among parenchyma cells, although some size decrease in surviving host wall appears to occur at times. Also, the epidermal cell walls are retained, even when the underlying parenchyma cells are completely broken down. Differences in reaction also occur between parenchyma cells. Parenchyma within the hymenium is entirely replaced by fungus tissue, whereas just below this area many host cell walls are retained. Near the lesion margin, an even greater difference in the interaction is apparent; when a change occurs in these host cells it mainly appears to be some shrinkage of the central vacuole, with a subsequent reorganization of the peripheral cytoplasmic components. Sometimes ultrastructural modifications occur in the organelles, but there is no evidence that the organelles are unable to function.

An alternative explanation for this host-parasite interaction is that it may not be a typical necrotic leaf spot disease, but may instead have attributes of diseases caused by biotrophic organisms. This interpretation would indicate that much of the necrosis occurs as a result of the fungus destroying host tissue in the area where the ascoma will develop. The lesion is described as having a dark, holonecrotic center surrounded by a yellowish, plesionecrotic area from which hyphae enter green leaf cells (36,41); although some necrosis occurs beyond the ascoma, hyphae do infect healthy looking host cells near the lesion margin. It is possible that food

may be obtained up through the time of sporulation from nutrients translocated to the ascoma through hyphae that extend into healthy green tissue. This source of nutrients was postulated by Morgan and Parbery (28), who found that the ratio of leaf weight to total plant weight is higher in diseased plants than in healthy plants, while the ratio of root weight to total plant weight is lower. They noted that the pathogen successfully competes for nutrients that would normally go to sinks such as the roots and lateral shoots. The conclusion they reached is that this interaction resembles that between biotrophic fungi and their hosts.

The inability of young infections to form ascomata on dead leaves in petri dishes provides evidence to support their hypothesis. Although ascomata developed in spots on living leaves, none formed on dead leaves, indicating that at least some contact with living tissue may be necessary for the fungus to mature.

Field studies with leaves in packets provided further information about whether ascomata can develop on dead leaves. During the growing season, ascospores were discharged from infected leaves that had mature (and younger) ascomata at the time of collection. However, mature ascomata did not seem to be able to form in leaves that initially had only young infections.

The results from overwintering leaves were somewhat different. Leaves bearing lesions ranging from immature spots to erumpent ascomata in the fall were capable of producing erumpent ascomata in the spring. These results were similar to those obtained from earlier studies that were primarily undertaken to determine how and where the fungus overwinters. Jones (16) and Schmiedeknecht (34,37) concluded that the fungus overwinters on diseased foliage that does not decay, and Schmiedeknecht (37) further noted that diseased leaves on the ground are an infection reservoir. However, Schmiedeknecht (34) determined that ascomatal primordia are the overwintering stage; ascomata with paraphyses, ascogenous hyphae, and some asci, but without ascospores, are formed in the fall, and serve as inoculum in the spring. Unlike the current study, ascospores have been obtained in the spring from ascomata in overwintered alfalfa leaves. Jones (16) placed ascomata that did not initially have ascospores into a moist chamber, where ascospore development was apparently induced. The energy for ascospore production in the spring may come from reserves that are stored in the overwintering ascoma, and not from nutrients obtained by the fungus from dead leaves.

The question of whether *Pseudopeziza* invariably causes necrosis of infected host cells, or whether it establishes a compatible relationship with its host, is not easily answered. Examination of the progress of the disease caused by *Pseudopeziza* on alfalfa indicates that the fungus may represent a group of organisms that is not readily placed into a specific category. However, this study indicates a number of reasons for the success of *Pseudopeziza* as a parasite.

The fungus is able to penetrate directly through the host cuticle and wall, eliminating the need to contact a particular portal of entry into the host.

The time needed for disease development is temperature dependent, but lesions have been reported to take 6–8 days to develop on leaves, and ascomata 2–4 weeks (16,19,39,40). Kristinsson (19) also noted that the mycelium is able to grow from the upper epidermis, through the parenchyma layers, and down to the lower epidermis by 10 days after infection. These times are similar to the results found in the present study, where visible leaf spots took at least 5 days to develop, and ascomata with ascospores about 2 wk. The fungus thus has a relatively short latent period.

Infection by the fungus causes host cell necrosis. Some of the necrotic host cells are replaced by the ascoma of the fungus, but the walls of the collapsed, infected host epidermis are retained after infection, as are the walls of some of the infected parenchyma cells. The fungus therefore begins sporulation inside the plant, remains within host tissues until the time of ascospore dispersal, and is consequently sequestered from the physical environment for a major portion of its life cycle. Exposure of the fungus only occurs during part of the inoculum production period, and during dispersal, germination, and penetration. The pathogen thus enters a niche inaccessible to many other fungi, eliminating some potential competitors.

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