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Ultrastructural Pathology of Cells Affected by *Pratylenchus penetrans* in Alfalfa Roots

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Abstract: Cortical parenchyma cells penetrated and fed upon by *Pratylenchus penetrans* for 48 hours contained only cytoplasmic debris. Proximal cells had an increase in tannin deposits, degenerated mitochondria, increased numbers of ribosomes, and no internal membrane structure. Often the endodermis was collapsed and contained massive tannin deposits on the inner cell wall and cell lumen. Similar observations were made in the stele, except tannin deposits were not as prominent. Multivesiculate structures were observed both in the endodermis and in the stele.

Key words: alfalfa, electron microscopy, *Medicago sativa*, *Pratylenchus penetrans*, root-lesion nematode, ultrastructure.

Most ultrastructural studies of the relationship between plant-parasitic nematodes and plant roots have focused on the root-knot (*Meloidogyne*) and cyst (*Heterodera*) nematodes which have complex and sophisticated interrelationship with their hosts (4,6,8). These two sedentary genera of nematodes induce the formation of feeding cells in their hosts. The root-lesion nematode (*Pratylenchus*), a migratory endoparasite, has a more primitive relationship with its host (4). In an earlier light microscopy study of forage legumes, histological and histochemical changes caused by *P. penetrans* were described (16). Discoloration of cells adjacent to a feeding nematode was associated with polyphenolic oxidation and concomitant tannin deposition. The most severe discoloration occurred in the endodermis and stele; however, *P. penetrans* was found only in the parenchymal cortex. The present study examined the ultrastructural changes induced by the feeding and migration of *P. penetrans* in the root tissues of alfalfa.

Materials and Methods

Pratylenchus penetrans (Cobb) Filipjev & Schurmans–Stekhoven was extracted aseptically from alfalfa callus tissue cultures in miniature Baermann pans (15) for 14 hours prior to their use as inoculum. The Cornell population used in this study originated from a single gravid female and has been monoxenically cultured since 1963 by W. F. Mai, Department of Plant Pathology, Cornell University, Ithaca, New York.

Alfalfa (*Medicago sativa* L. cv. Du Puits) seeds were surface sterilized in 37 N sulphuric acid for 30 minutes, washed in cold sterile water, and germinated in inverted petri dishes of sterile water agar. Seedlings, 72 hours old with 20-mm-long roots, were inoculated with *P. penetrans*.

Aseptic alfalfa seedlings were placed equidistant on the surface of sterile water agar in 90-mm-d petri dishes (five seedlings in each of 10 dishes). Two microbiologically sterile females of *P. penetrans*, hand picked with the aid of a dissecting microscope, were placed in a drop of sterile water introduced at the root-hair zone. Each inoculated root was covered with sterile silica sand and moistened further if necessary. The nematodes were allowed to penetrate and feed in the roots for 48 hours. Roots were then fixed and embedded. Uninoculated seedlings were prepared for controls.

Lesioned segments, 2 mm long, were excised from infected roots and fixed for 2½ hours in 5% glutaraldehyde, pH 6.8, at room temperature. Root segments were then washed in 0.2 M cacodylate buffer, pH 7.2, for 2 hours at 5 C with buffer changes every 10 minutes. The segments were rinsed in distilled water for 5 minutes and postfixed in 2% osmium tetroxide for 20 minutes, both at 5 C. The segments were dehydrated in an acetone series from

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25 to 70% at 5 C and from 90 to 100% at 25 C. Tissue segments then were passed through an epoxy : acetone (v/v) gradient and embedded in pure epoxy resin. Uninoculated root segments were processed in the same manner. Light and electron microscope sections, 1 μm and 500 nm, respectively, were cut with a Sorval MT2-B ultramicrotome (Sorval Porter Blum, Newtown, CT) and stained in 2% uranyl acetate, followed by Reynolds lead citrate (13), and examined in a Philips 201 electron microscope.

Results

Within 48 hours of inoculation, a yellowish elliptical lesion, ca. 1 mm long, developed where a nematode had penetrated a root. The long axis of the lesion was parallel to that of the root. Dark brown cells appeared in the centers of some of the lesions at 48 hours.

Light micrographs of cross sections of root tissues revealed *P. penetrans* within the cortical parenchyma (Fig. 1). In all cross sections examined, the nematode always was found in the cortex. Necrosis and cell collapse were observed in the cortex in relation to nematode activity (Fig. 2). The cytoplasmic content of both the cortical parenchyma and endodermal cells darkened (Fig. 2). Cells surrounding the feeding nematode appeared to have thickened walls, perhaps resulting from the apposition of condensed cytoplasm (Fig. 2).

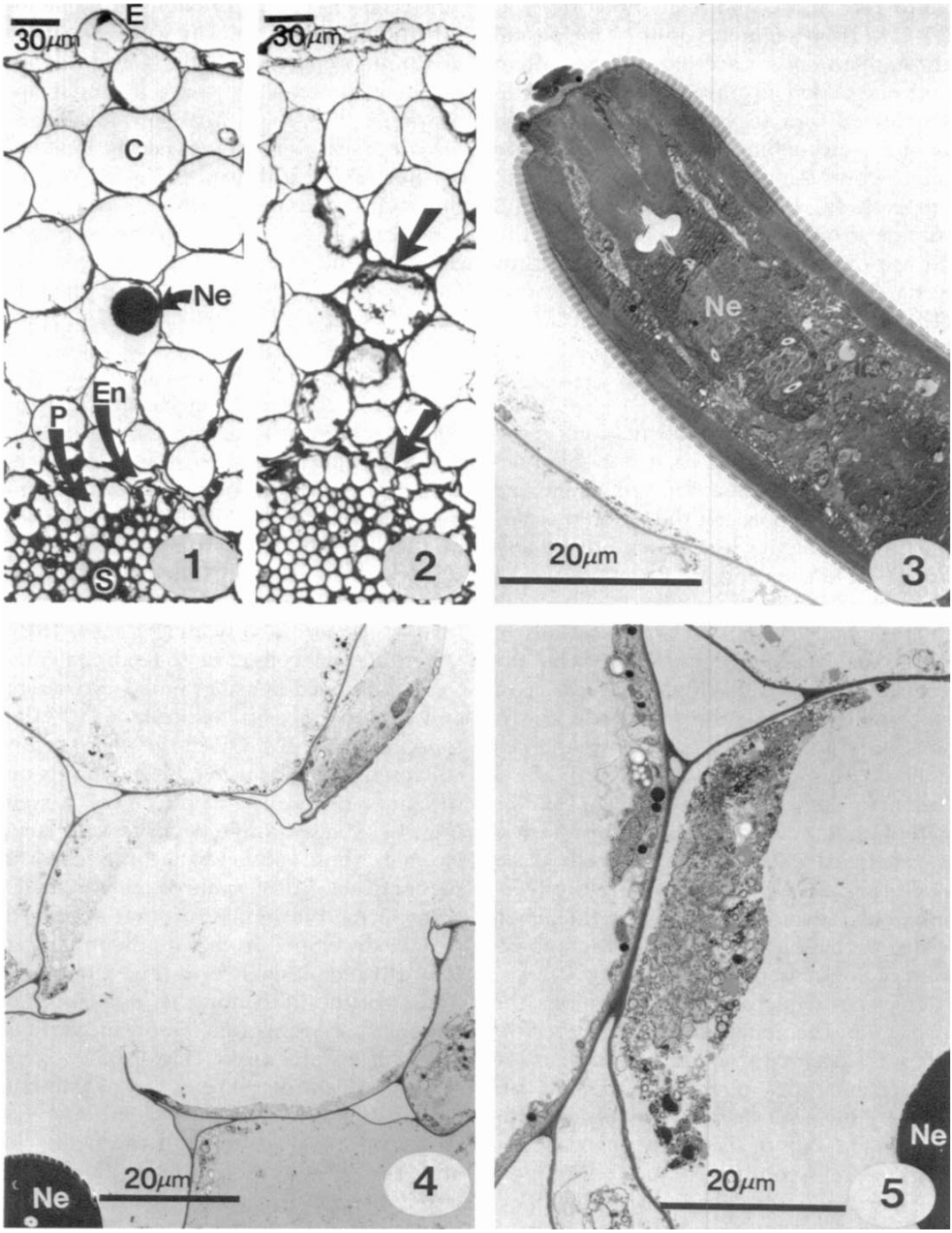
In electron microscopic observations, the passage of the nematode through the cortex was observed to be intracellular (Figs. 3, 4). Nematodes were usually aligned parallel to the stele as they penetrated the transverse walls of successive cortical cells. Cortical cells penetrated and fed upon by the nematode were generally devoid of cytoplasmic content (Fig. 4). The remaining cytoplasm lacked integrity without recognizable organelles or membrane structure (Fig. 5).

Cortical cells adjacent to cells penetrated and fed upon by the nematode contained numerous electron dense osmiophilic deposits both in the cytoplasm and in the vacuole (Figs. 7–11). In previous histochemical studies, these deposits were shown to be tannin depositions (16). Tannin depositions in other tissues have a similar appearance (2,9). By comparison, fewer tannin deposits were observed in healthy, uninfected cortical tissues (Fig. 6). Within the vacuole, tannin deposits appeared as spherical droplets affixed to the tonoplast (Fig. 7). In other affected cortical cells, the tannin deposits formed a dark, densely staining layer on the tonoplast (Figs. 8, 9).

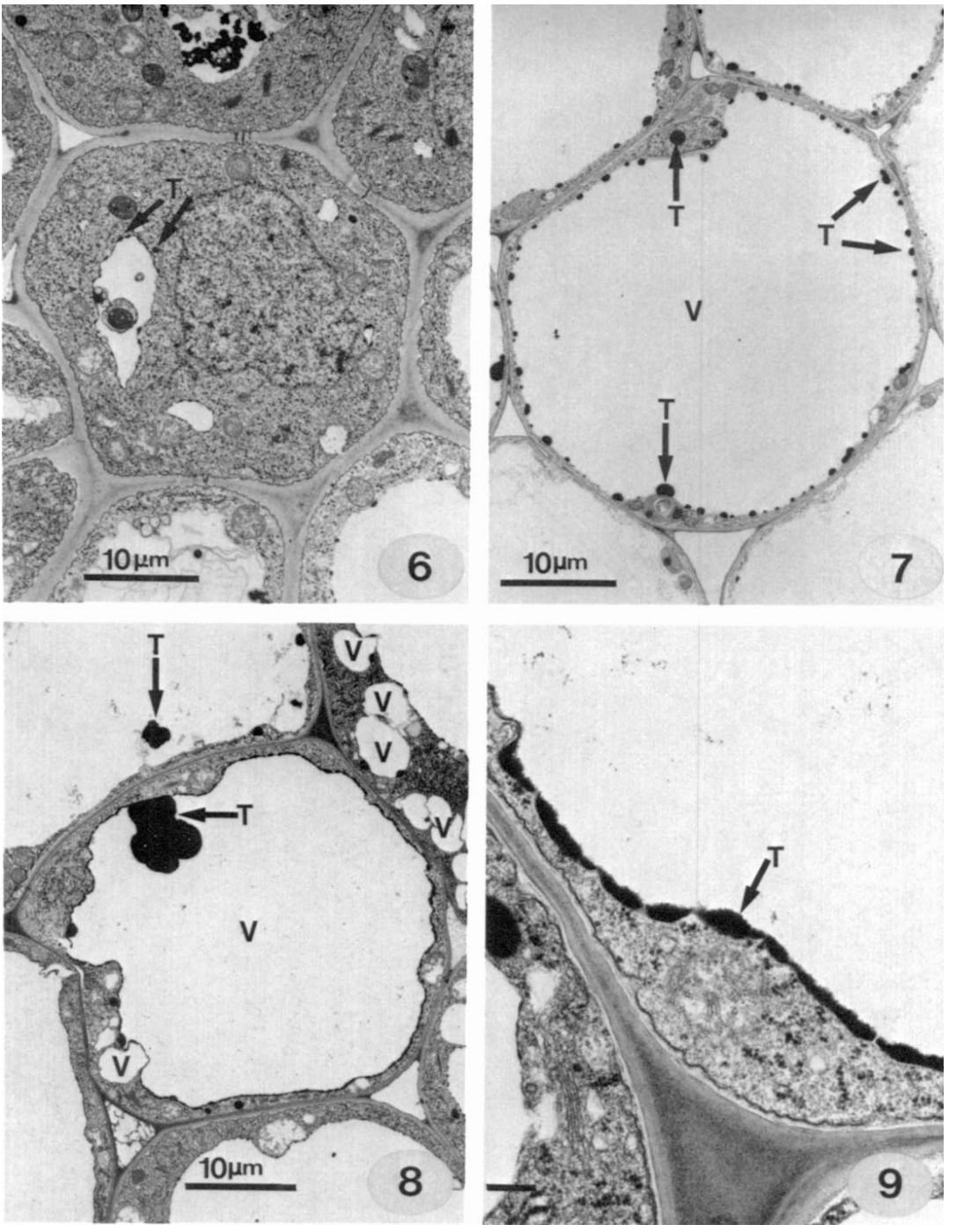
Cortical cells adjacent to migrating and feeding nematodes had swollen and distorted mitochondria that lacked clear internal membrane structure (Fig. 13). Also, the density of ribosomes increased noticeably in comparison to cortical cells in uninfected root tissue (Fig. 12). Membranes in affected adjacent cells showed varying stages of degeneration. The plasmalemma had retracted from the cell walls and formed numerous vesicles (Figs. 14, 15).

Endodermal cells close to feeding nematodes contained dense granular cytoplasm and tannin deposits associated with the tonoplast (Fig. 16). Often the endodermis collapsed and had large tannin deposits on the inner cell walls and in the cell lumen (Fig. 17). These tannin deposits were larger than those observed in the cortical parenchyma. Also, multivesiculate structures formed invaginations that appeared to have developed from both the tonoplast (Fig. 18) and plasmalemma (Fig. 19). Membranes of the mitochondria, nucleus, and endoplasmic reticulum were in various stages of disintegration (Fig. 18).

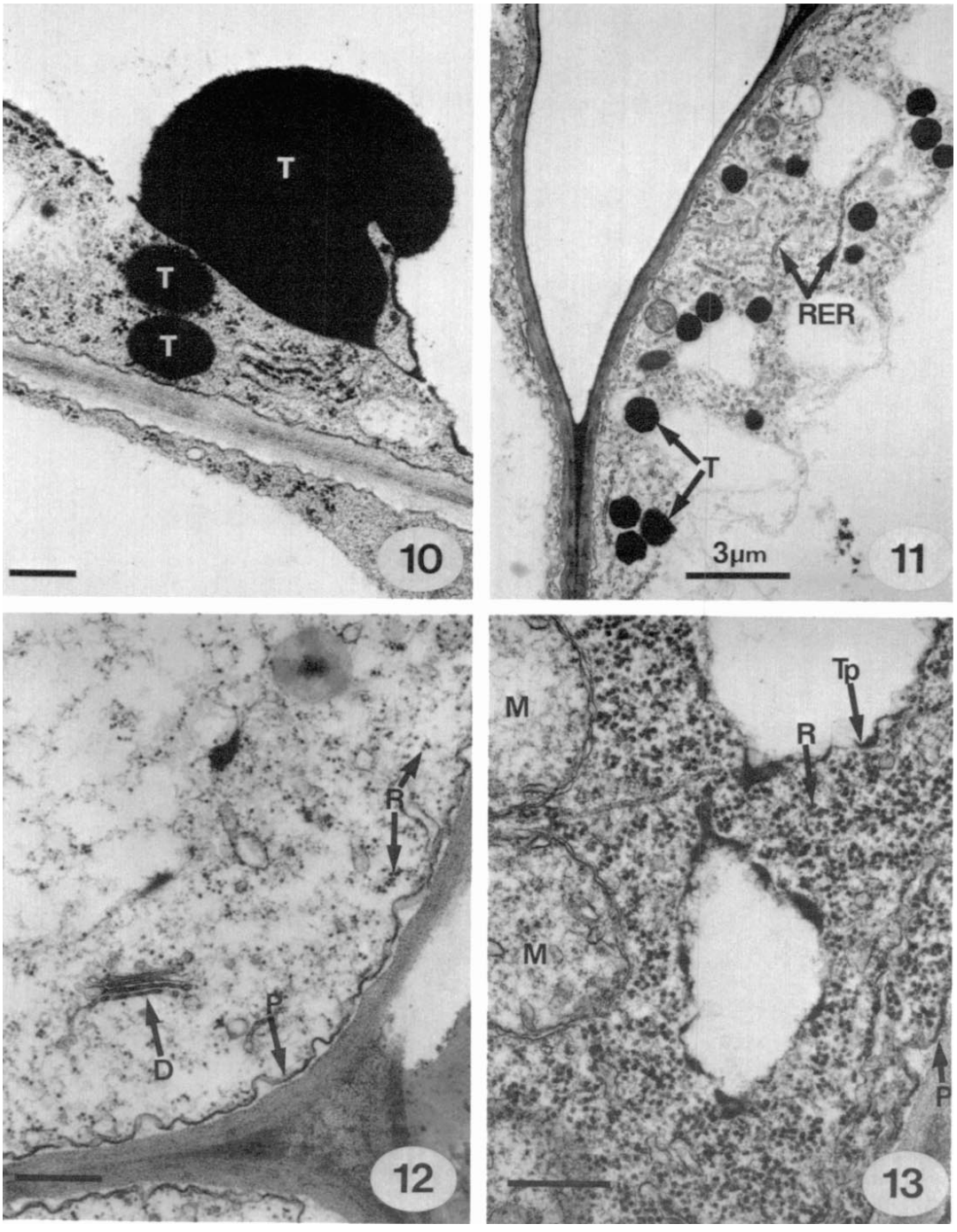
Tannin deposits were not as prominent in the stele (Figs. 21–23). Membrane vesiculation occurred in both the pericycle and vascular cells (Figs. 20, 24, 25). In the latter, the cytoplasm was granular, disorganized, and in various stages of collapse (Fig. 21). Tannin depositions lined the tonoplast as large discrete deposits (Figs. 22, 23). Membranes of the endoplasmic reticulum, dictyosomes, mitochondria, and nucleus were swollen and in various stages of degeneration (Figs. 24, 25). Ribosomes frequently increased in these cells (Fig. 25).



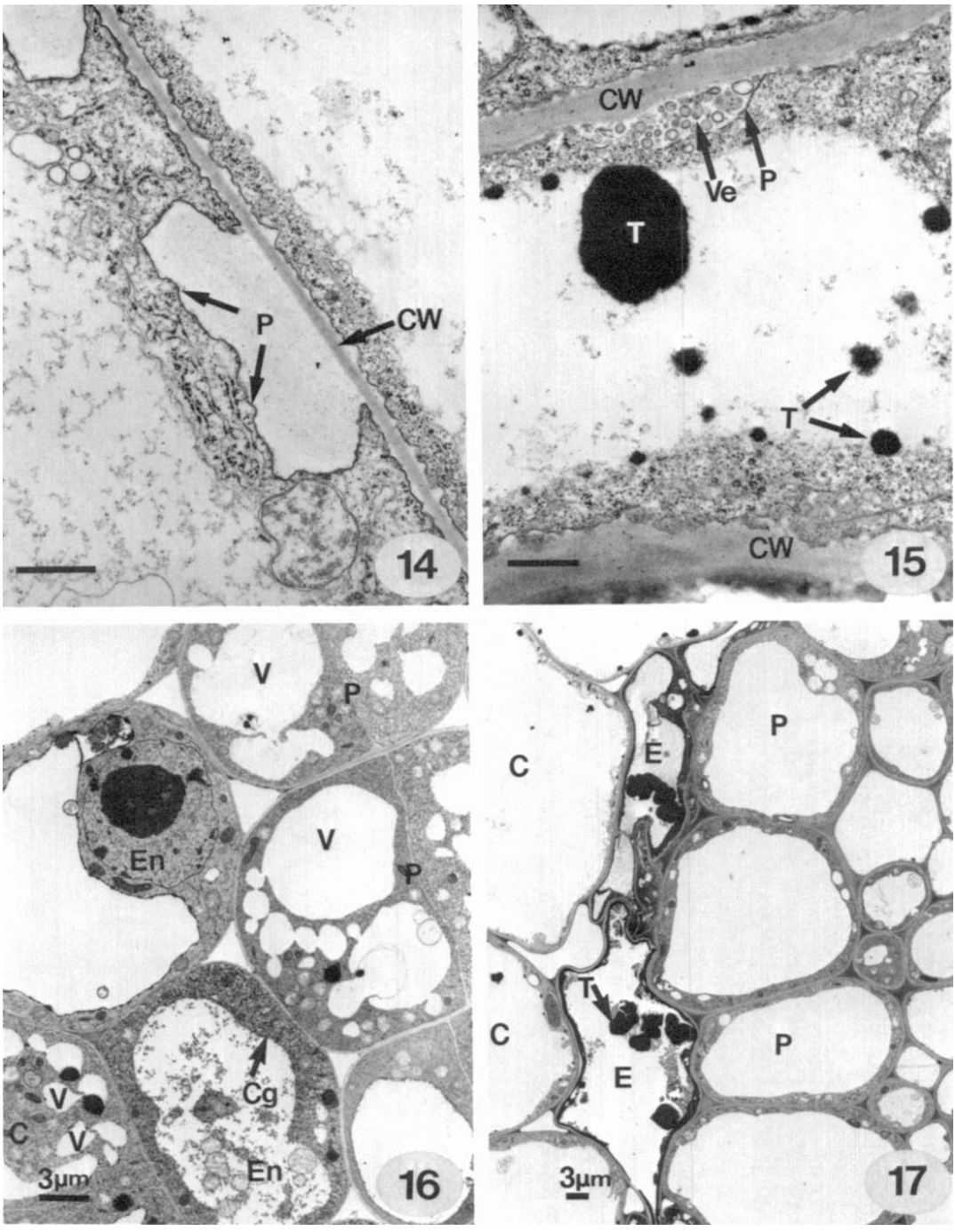
FIGS. 1–5. Light and electron micrographs of *Pratylenchus penetrans* in the cortex of alfalfa roots. 1) *P. penetrans* in cortical parenchyma (C). E = epidermis; En = endodermis; P = pericycle; S = stele; Ne = nematode. 2) Discoloration and collapse of cytoplasm caused by *P. penetrans* in cortical parenchyma. Note apparent thickening of cell walls in cortex and endodermis (arrows). 3) Longitudinal section of *P. penetrans* in a cortical parenchyma cell. 4) Broken cortical cell walls following nematode feeding and passage. Note that cells are generally devoid of cytoplasm. 5) Condensed cytoplasm in cortical cell fed upon by *P. penetrans.* Note lack of cytoplasmic integrity and absence of membrane structure.



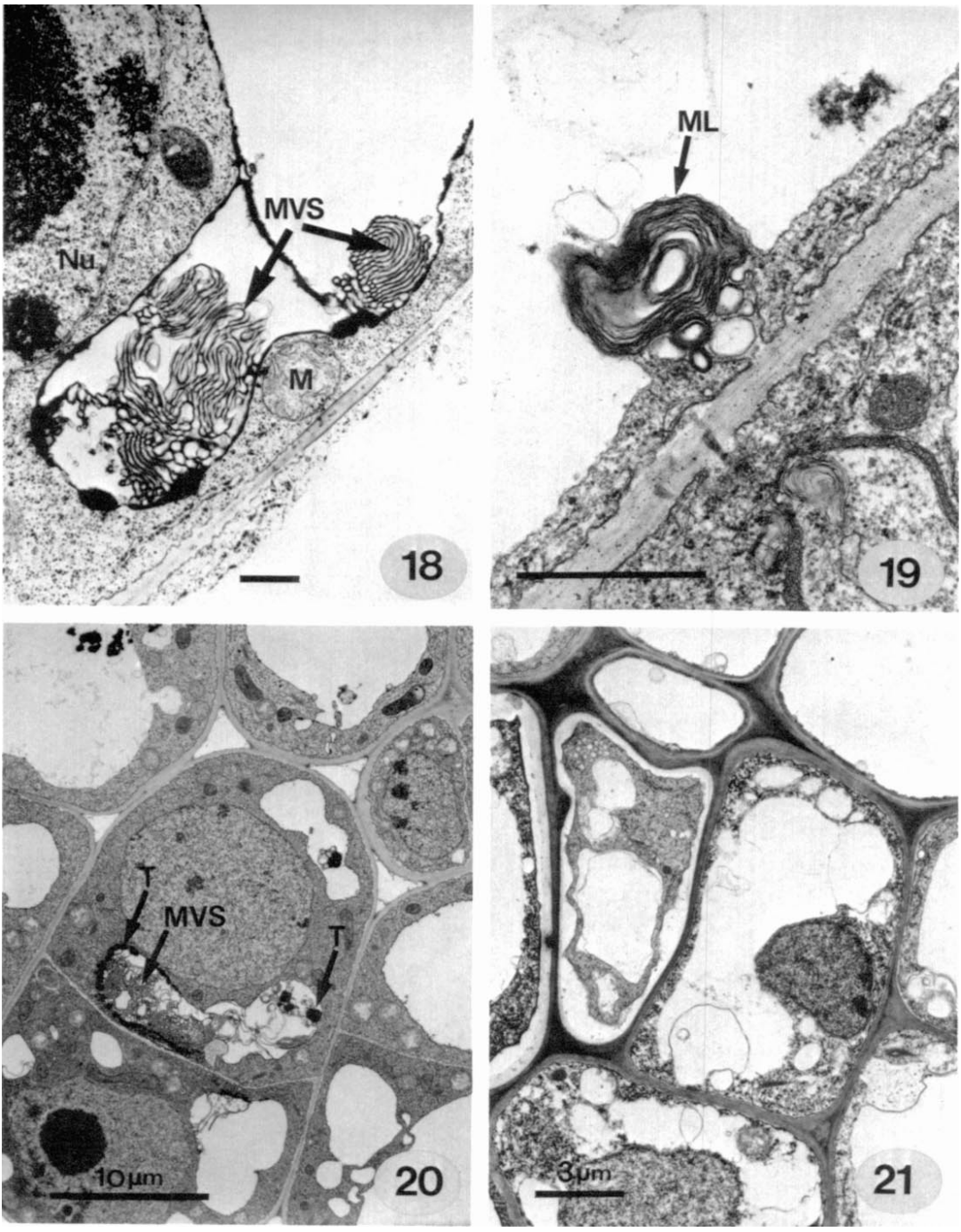
FIGS. 6–9. Depositions in cortical parenchyma. 6) Healthy cortical cell in uninoculated root. Note low level of tannin deposition (T) in cells. 7) Tannin deposits in cytoplasm and vacuoles (V) of cortical cells adjacent to nematode feeding sites. 8, 9) Tannin deposits on the tonoplast of affected cortical cells. Note increased cytoplasmic vacuolation. Unlabelled bars = 5 μm.



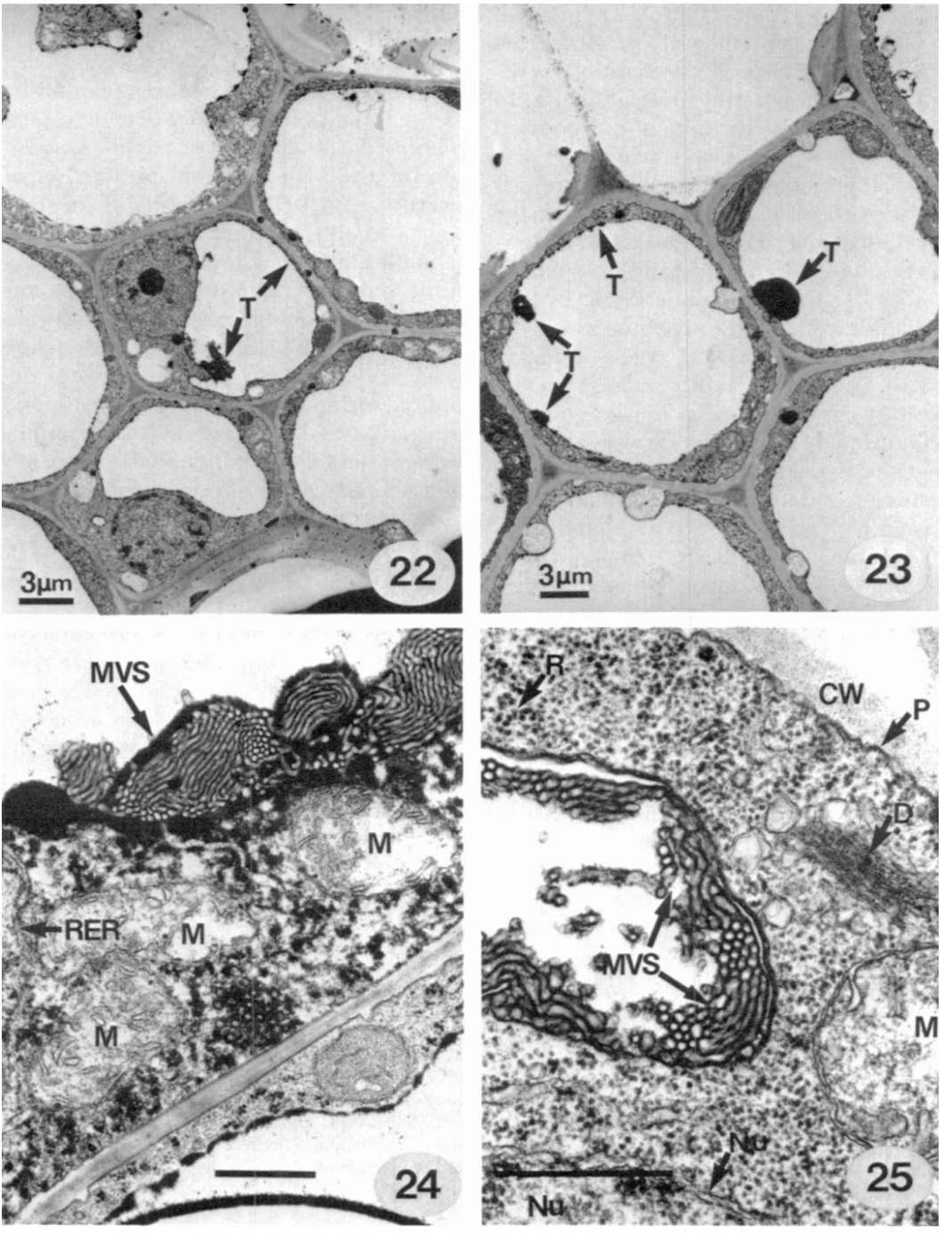
FIGS. 10–13. Cytoplasmic changes in cortical cells affected by *P. penetrans*. 10, 11) Increased tannin (T) deposition in cytoplasm in cells adjacent to nematode feeding sites. Note abundant rough endoplasmic reticulum (RER). 12) Healthy cortical cell in uninoculated root. D = dictyosome; P = plasmalemma; R = ribosomes. 13) Cell bordering nematode feeding site. Note swollen mitochondria (M) with loss of internal structure and increased ribosome density. Both plasmalemma and tonoplast (Tp) are swollen and diffuse. Unlabelled bars = 5 μm.



FIGS. 14–17. Alfalfa root cortical cells adjacent to cells invaded by the nematode. 14, 15) Invagination of plasmalemma into the cytoplasm and increased vesiculation. CW = cell wall; Ve = vesicles; T = tannin. Note numerous vesicles between cell wall and swollen disintegrated plasmalemma in Figure 15. 16) Granular cytoplasm (Cg), and tannin deposit in endodermal cells adjacent to nematode feeding. Note increased vesiculation in endodermis (En) and pericycle. V = vacuole; C = cytoplasm. 17) Collapsed endodermal cells (E) with heavy tannin deposits on cell walls and in lumen. Unlabelled bars = 5 μm.



FIGS. 18–21. Endodermis and stele cells affected by nematode activity. 18) Multivesiculate structures (MVS) associated with the tonoplast. Nu = nucleus; M = mitochondria. 19) Concentrically arranged membranous lamina (ML) which appears to have originated from the plasmalemma. Figures 18 and 19 are endodermal cells. 20) Multivesiculate structures and tannin (T) deposits in pericycle. 21) Cytoplasmic disorganization in the stele. Note cytoplasmic collapse and increased vesiculation. Unlabelled bars = 5 µm.



FIGS. 22–25. Cytoplasmic degeneration and multivesiculate structures (MVS) in the stele. T = tannin. 22, 23) Tannin deposits on the tonoplast and in the vacuole. 24, 25) Membrane deterioration of rough endoplasmic reticula (RER), dictyosomes (D), mitochondria (M), nucleus (Nu), and plasmalemma (P). Note numerous ribosomes (R). M = mitochondria. Unlabelled bars = 5 µm.

Discussion

This is the first study of the ultrastructure of the host–parasite relationship between *P. penetrans* and roots of alfalfa. In light microscopy investigations reported earlier (16), the physical activity of *P. penetrans,* as previously reported, was confined to the cortical parenchyma. The damage to cells in which the nematode had fed was the result of both physical and biochemical factors (1,16). Cells penetrated by the nematode contained only cytoplasmic debris which was composed largely of degenerated organelles and condensed cytoplasm. Cells proximal to the line of cells penetrated by the nematode were typically degenerative and characterized by darkly staining condensed and vesiculate cytoplasm which was appressed to the cell walls.

Common ultrastructural changes were seen in the cortical parenchyma, endodermis, pericycle, and vascular cells. Such changes included condensed necrotic cytoplasm; increased tannin depositions in the cytoplasm, on the tonoplast, and in vacuoles; loss of membrane integrity in the plasmalemma, tonoplast, and nuclear membrane; and degeneration of the mitochondria, dictyosomes, and endoplasmic reticula. Increased vesiculation and the formation of multimembrane inclusions were seen only in the endodermis, pericycle, and vascular cells. These myelin-like inclusions were found within the cytoplasm and vacuoles and, as suggested by Baur and Walkinshaw (14), apparently are formed by endoplasmic reticulum activity or by infolding of the tonoplast.

Previous light microscope studies detected tannin-like deposits in cells affected by nematode activity (16). Electron microscope studies revealed tannin deposits in central vacuoles and large cytoplasmic vesicles. Massive cellular deposits of tannin were usually associated with cytoplasmic disorganization and granularization and lack of membrane integrity. The intense tannin deposits and concomitant collapse of the endodermis may present a barrier to nematode entry into the stele of alfalfa. β-glucosidase, secreted by *P. penetrans,* has been implicated in the release of phenols bound up in phenolic glycosides (11,12). Subsequent oxidation of these phenols by cytoplasmic polyphenoloxidases has been identified as the principle factor responsible for the browning and production of necrotic tissues (1). Formation of free tannin molecules in cytoplasm has been causally implicated in the precipitation of structural and enzymatic proteins (2). In our electron microscope observations, degeneration in cells was observed only when large concentrations of tannins were deposited within the cells or when membrane integrity was lost. Low levels of tannins were observed in healthy alfalfa root cells and no deleterious effects were seen. The apparent thickening of cell walls, observed in light microscopy studies (16), appears to be the result of the deposition of condensed granularized cytoplasm against the cell walls. Such deposits, usually adjacent to nematode feeding sites, are likely associated with lignin-like substances (6). Host localization defenses, sometimes effective against slower penetrating plant pathogens such as fungi (10), are often causally associated with the effects of phenolic oxidation. This type of host reaction does not appear to impede feeding and migration of *P. penetrans* throughout the root cortex of alfalfa.

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