Some Ultrastructural Changes Induced in Resistant and Susceptible Soybean Roots Following Infection by Rotylenchulus reniformis

R. V. REBOIS, PHILIP A. MADDEN, and B. JOE ELDRIDGE[1]

Abstract: A developmental electron microscopic study of the parasitism of *Rotylenchulus reniformis* in resistant 'Peking' and susceptible 'Lee' soybeans was made during a 21-day period under controlled conditions. Within 2 days of inoculation, the nematode had penetrated the cortical cells to the endodermis where it inserted its stylet, secreted and initiated syncytial formation and cell hypertrophy. Syncytia primarily involved pericycle tissues and, to a lesser extent, xylem parenchyma and endodermis. When identifiable, the cell into which the nematode stylet was inserted to initiate syncytial development was endodermal. Susceptible tissues exhibited two basic phases of development during this infection period: (i) an initial phase represented by partial cell wall lysis and separation; and (ii) an anabolic phase, characterized by organelle proliferation and development accompanied by secondary wall deposits, which provided nutrition for sessile female development. The resistant or hypersensitive reaction (HR) lacked the anabolic phase found in the susceptible reaction, and was characterized by an extension and usually accelerated type of lysis found in the first phase of the syncytial development. The HR was usually very evident 4 days after inoculation, and could be identified by an almost complete lysis of the cell walls and cytoplasm. The possibility that the initial cell of the developing syncytium or "prosyncyte" may influence a susceptible or resistant reaction is discussed. Successive stages of cell wall dissolution and the deposition of secondary cell walls are described. *Key Words:* reniform nematode, *Glycine max*, syncytia, cell wall, boundary formation, wall lysis, wall deposits.

Light-microscopic histopathology of *Rotylenchulus reniformis* Linford and Oliveira 1940 on resistant 'Peking' and susceptible 'Lee' soybeans, *Glycine max* L. Merr, cultivars has been reported (28). Studies on the histopathology of *R. reniformis* on other crops using light microscopy are documented (1, 2, 5, 20, 21, 30). Light-microscopic (7, 8) and electron-microscopic investigations on *Heterodera glycines* (12, 13, 29) parasitism of Lee and Peking are pertinent to the present study because both *R. reniformis* and *H. glycines* females parasitize similar root tissues in both cultivars. Furthermore, Lee is susceptible to both nematode species, while Peking has a high degree of resistance to *R. reniformis* (28) and to most isolates or strains of *H. glycines* (18). This work was undertaken to determine the ultrastructural and physiological changes which occur in *R. reniformis*-resistant and -susceptible soybean cultivars, and to gain some insight on the biosystems and timing

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involved in these reactions. Preliminary electron microscopy on this subject was recorded (26).

Recent review articles by Dropkin (6), Endo (9), and Webster (33) provide background information on other sedentary nematodes which, like *R. reniformis,* (28) are known to induce syncytia and may cause hypersensitive responses in plants.

MATERIALS AND METHODS

Unless otherwise indicated, all equipment and material was autoclaved for 15 min at 1.05 kg-force/cm (15 psi), and sterile transfer techniques were used.

R. reniformis-susceptible Lee and -resistant Peking soybean seeds were surface sterilized with a 1-min rinse in 95% ethanol followed by a 30-min dip in 1% sodium hypocholorite solution. The seeds were transferred to petri dishes containing potato-dextrose agar (PDA) and incubated for six days at room temperature. Axenic appearing seedlings (those without visible signs of microbial contamination) were transferred from PDA to French square jars containing fine vermiculite that had been moistened with 1/3-strength Hoagland's nutrient solution number 1 (14). Each jar contained one seedling, was covered with a thin polyethylene membrane, and supported in a 29 C water bath (27). A 16-h day was maintained using fluorescent and incandescent lights. Three days after the seedlings were transplanted, each jar received either an aqueous aliquot containing 3,000 surface-sterilized nematodes, or an equivalent amount of supernatant. Nematodes were sterilized by the Peacock method (23) followed by two sterile water rinses, 48-h in a sterile solution containing 50 mg of streptomycin sulfate and 20 mg of quinolinol sulfate per liter of water, and two final rinses in water before they were added to the vermiculite. Nonsterile nematodes were also introduced into some jars to determine the effect of the sterilization technique on pathogenicity. After the seedlings were exposed to nematodes for two days, they were removed from the vermiculite and rinsed three times in sterile water to remove any unattached females before being transferred to sterile jars containing fresh vermiculite and nutrients. At this time, 100 surface-sterilized *R. reniformis* males were added to three jars of each cultivar, since males reportedly are necessary for large egg production (19). Just before harvest, vermiculite from each jar was aseptically placed on PDA to verify that gnotobiotic conditions had been maintained. At 2, 4, 9, 16, and 21 days after inoculation the roots were washed in tap water, excised in fixative, fixed for 4-24 h in 3% glutaraldehyde in 0.05 M potassium phosphate buffer at pH 6.85, rinsed 1-h in six changes of 0.05 M buffer, and post-fixed for 2-h in 2% osmium tetroxide in 0.05 M buffer. Specimens were dehydrated in acetone and embedded in low-viscosity resin according to Spurr (31). Silver to grey sections (40-70 nm) were cut with a diamond knife, mounted on uncoated 75 × 300 μm (opening size) copper grids, and stained with uranyl acetate and lead citrate for examination with an AEI EM 6B microscope at 60 KV. For light-microscopic studies and orientation, sections 0.5 - 1.0 μm thick were mounted on glass slides and stained with alkaline toluidine blue. A few specimens used in this study were grown and inoculated by methods outlined previously (26), killed and fixed as described above, dehydrated in an ethanol series, transferred to propylene oxide, embedded in Maraglas, mounted on 75 × 75 μm (opening size) formvar coated grids, and examined with a Philips 300 electron microscope at 40-60 KV.

RESULTS

R. reniformis were observed on all parts of the soybean seedling roots, but mainly on the lateral roots from which most samples were taken. From one-to-five nematodes were observed parasitizing the same site. Sections also were taken from parasitized areas nearest the root tips to determine if the root meristem was parasitized or reacted differently from differentiated tissues; however, root differentiation and root hairs were already evident in these sections (1-2 mm behind the root tip) within the first 2 days of inoculation.

Susceptible reaction: Within 2 days of inoculation, the infective female had penetrated the root cortex intracellularly to the stelar region. At points of nematode penetration, cortical cell walls had jagged torn edges, were folded centripetally, and formed a partial stricture around the nematode body (Fig. 1). When the nematode became sedentary and swollen, body constrictions

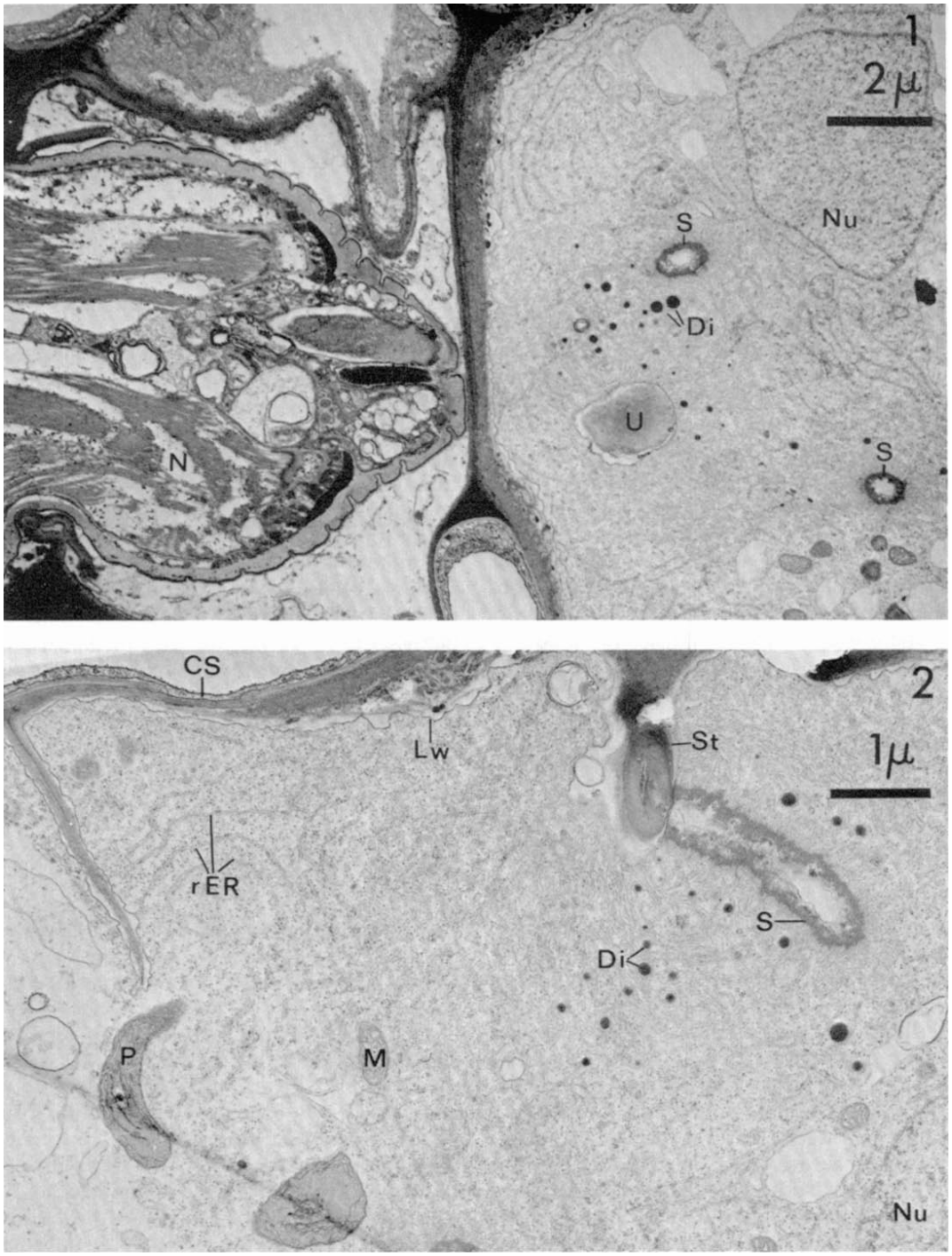


FIG. 1-2. **1)** Nematode head embedded in cell showing torn, penetrated cell walls and constricted nematode body in 'Lee' soybean two days after inoculation. Nematode lips are appressed against cell wall in juxtaposition with an endodermal cell which shows some wall lysing. Nematode excretion is evident and associated with dense inclusions which resemble secretion granules or possibly hydrolytic reaction products. **2)** Stylet inserted into an endodermal cell identified by Casparian strip. Wall near stylet is lysing in 'Lee' soybean two days after inoculation, while separated vestigial wall in advance stage of lysis separates original pericycle from endodermal cell. The plastid suggests that cytoplasmic flow is away from the stylet. CS = Casparian strip, Di = dense spherical inclusions, Lw = lysing wall, M = mitochondrion, Nu = nucleus, N = nematode, P = plastid, rER = rough endoplasmic reticulum, S = secretion or exudate, St = stylet, U = unidentified body which may be a nematode exudate.

were formed at the points of contact with the penetrated cell walls. These constrictions remained evident after the females were dislodged from the root. Cortical cells through which the nematode passed were disorganized and usually devoid of protoplasm (Fig. 1). Their walls were slightly thickened and stained readily with toluidine blue which produced mostly a green color when observed with light microscopy. Body penetration apparently stopped when the nematode's head reached the endodermis (Fig. 1, 2, 4). The stylet was inserted into the endodermal cell which apparently became the initial syncytial cell. The term "prosyncyte" is proposed and used in this text to designate that particular cell where nematode migration stops and the stylet and/or possibly the labia are inserted to initiate syncytial formation and sessile feeding. It is also the first cell included in the syncytium whether the syncytium be formed by endomitosis without cytokinesis and/or a coalescence of the cytoplasm of adjacent cells without karyokinesis. It is presumed that nematode secretion(s) from the stylet helps initiate rupture of the prosyncyte wall into adjacent pericycle cells (Fig. 2, 4). Only one endodermal cell, the prosyncyte, appears to be involved in early syncytial development which is made up mainly of parenchymal cells of the pericycle radiating from it. The nature of the material extruded from the stylet (Fig. 3) is unknown but will be referred to as excretion or secretion, interchangeably. The stylet excretion pictured appears to diffuse and cause eddys where it contacts endoplasmic reticulum and other cytoplasmic components. Associated with these eddys were several small electron dense spherical inclusions or granules composed largely of particles about the size of ribosomes. Unidentified bodies, like the one pictured in Figure 1, were seen only in developing syncytia near the nematode's stylet.

For the purpose of direct comparisons, similar root tissues are presented in Figures 4 and 5, which were taken at the same magnification from opposite sides of the same thin section. This root parasitized by one female, was representative of infected (Fig. 4) and uninfected (Fig. 5) tissues during the early stages of parasitism. Partial dissolution and breakdown of cell walls in the pericycle and the successive stages by which it progressed during the first two days following infection are depicted in Figures 4, 6, and 7. Early cell wall lysis appeared as an amorphous, mostly electron-transparent, irregular thickening of the wall containing a few electron-dense bodies, some with less-dense matrices, along with what appeared to be fragments of primary cell wall (Fig. 6, top). As wall lysis progressed the amorphous area expanded, showed varying electron densities, and the primary wall became more or less rounded, not jagged, where separation was noted (Fig. 6, bottom). At this point the plasmalemma appeared to be constricting and/or mending about the wall break. Finally the cell wall parted and the amorphous lysing materials were presumably shed or passed through the plasmalemma to be absorbed in the cytoplasm (Fig. 7). Also, at the early stages of syncytial development, the unparted cell walls of the pericycle appeared to be stretched and longer than those of corresponding normal cells (Fig. 4, 5). Once parted, the total measured length of cell wall fragments between pericycle cells (Fig. 4) was generally a little less than that of normal pericycle cells (Fig. 5).

Other cytological changes noted in tissues during the first two days after infection were: early breakdown of the tonoplast; formation of many small vacuoles; slight increase in the numbers of mitochondria and dictyosomes, and increased numbers and size of plastids; increased density of cytoplasm together with an increase in rough endoplasmic reticulum and numbers of ribosomes; and a slight increase in nuclear size. There is very little evidence that cells surrounding the infected pericycle tissues were affected except for the crushing of a few endodermal cells near the feeding site. Also, a small amount of cell wall lysis was observed in endodermal cells adjacent to the prosyncyte.

Between 2 and 4 days after infection the syncytial cells appeared to shift into a highly anabolic metabolism. Cell walls and fragments thereof delineated most of the original cells and showed signs of thickening through secondary deposits (Fig. 8). Small protuberances were seen on cell walls and wall fragments within syncytial cells (Fig. 8, 15, 16). Cell wall dissolution was considerably reduced and almost absent, except for some small wall fragments near the nematode feeding site and a few cells located at distal parts of the syncytium. Many boundary

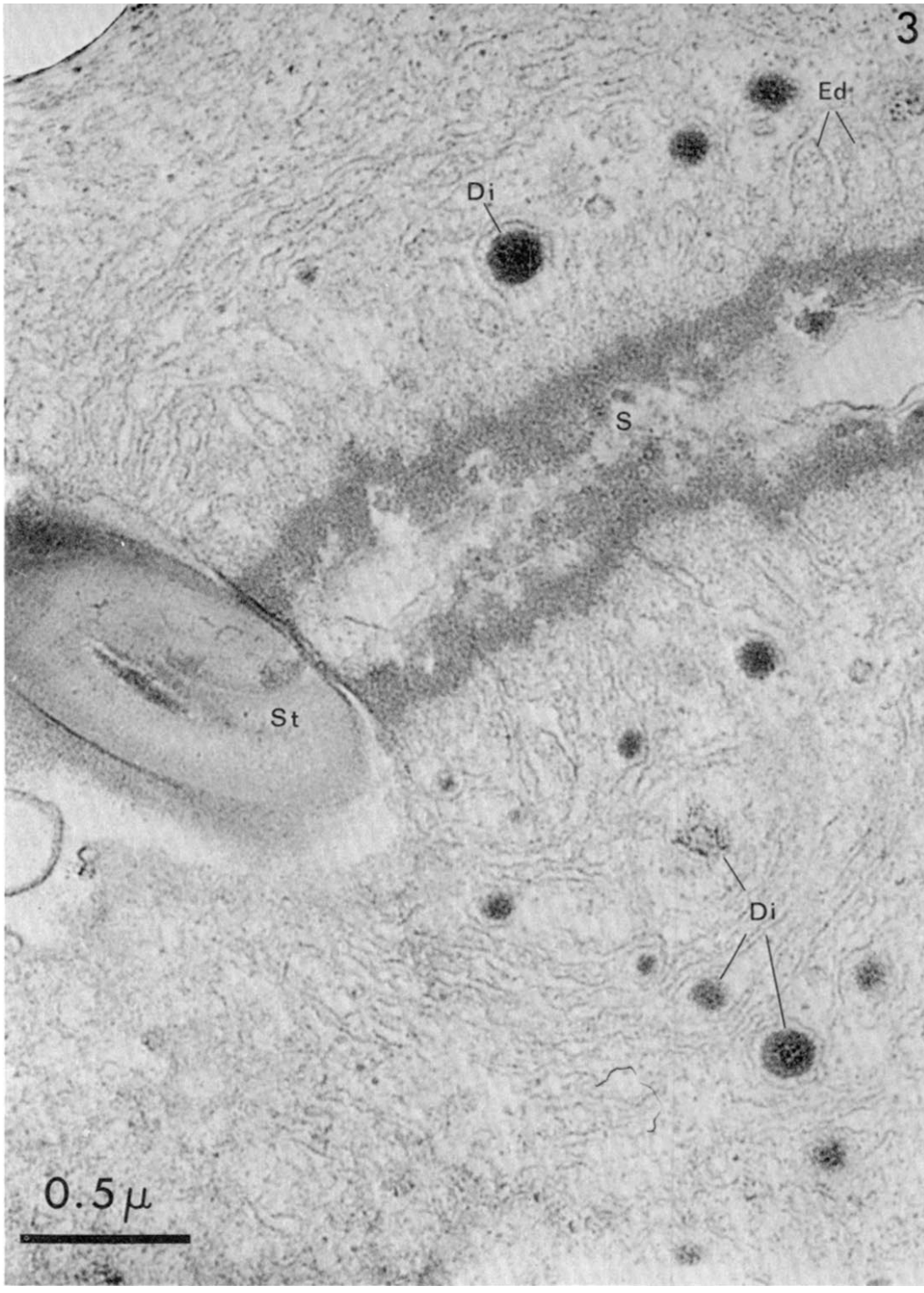


FIG. 3. Enlargement of nematode stylet and secretion (from portion of Fig. 2). Shows spherical inclusions of varying densities associated with the eddying front where the nematode excretion and adjacent cytoplasmic components meet. Dense spherical inclusions and secretion contain many particles which are about 10-20 nm in diam. The secretion has a less dense matrix. DI = dense spherical inclusion, Ed = eddying front, S = nematode secretion, St = stylet.

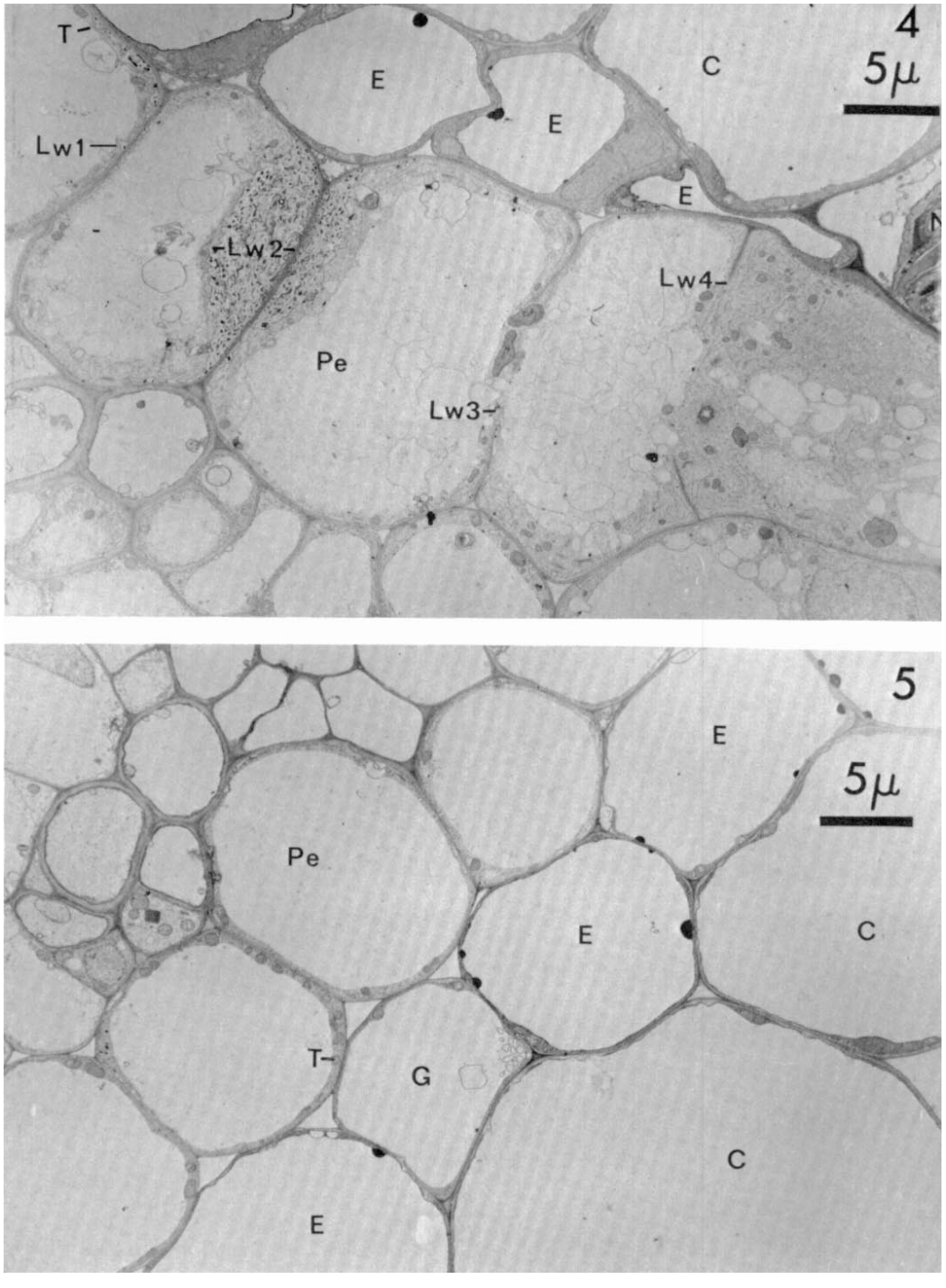


FIG. 4-5. 4) 'Lee' soybean two days after inoculation showing the initial syncytial cell or "prosyncyte" and the partial lysis stage of syncytial formation on a root parasitized by a single nematode. From left to right showing; early lysis (Lwl), thickened swollen wall in advanced stage of lysis (Lw2), parted cell wall showing reduced lysis with the plasmalemma continous about it (Lw3), and the vestigial lysed walls (Lw4). Also shown are early successive stages of central vacuole breakdown and the appearance of numerous small vacuoles. 5) Same magnification and section as Fig. 4, but showing similar healthy tissue at the opposite side of thin section. C = cortical cell, E = endodermal cell, G = possible gap or passage cell, Lw = lysing wall, N = nematode, Pe = pericycle cell, T = tonoplast.

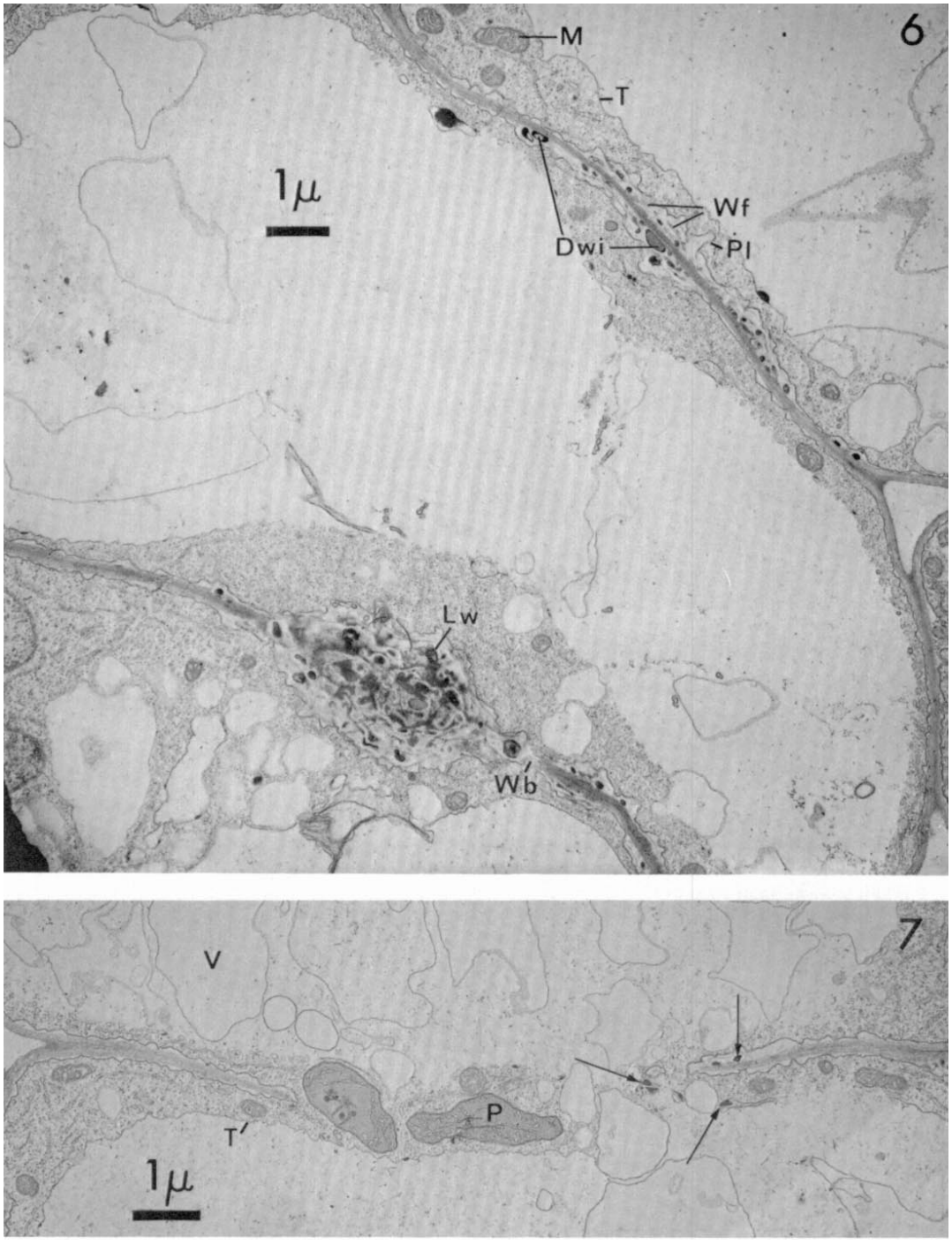


FIG. 6-7. 'Lee' soybean two days after inoculation showing successive stages of cell wall lysis. **6)** Top shows stretched wall in early stages of lysis with irregularly shaped clear area pushing the plasmalemma deep into the cytoplasm. Dense wall inclusions, some with less-dense matrices, and wall fragments are shown. Bottom wall is in a state of lysis and separation. 7) Wall shown after most of the lysis has taken place and wall separation is complete. Note the dense particles inside and out of the plasmalemma near the right wall break (arrows) indicating that lysed wall material may have been released into the cytoplasm. Dwi = dense wall inclusions, Lw = lysing wall, M = mitochondria, P = plastid, Pl = plasmalemma, T = tonoplast, Wb = wall separation occurring, Wf = primary wall fragment.

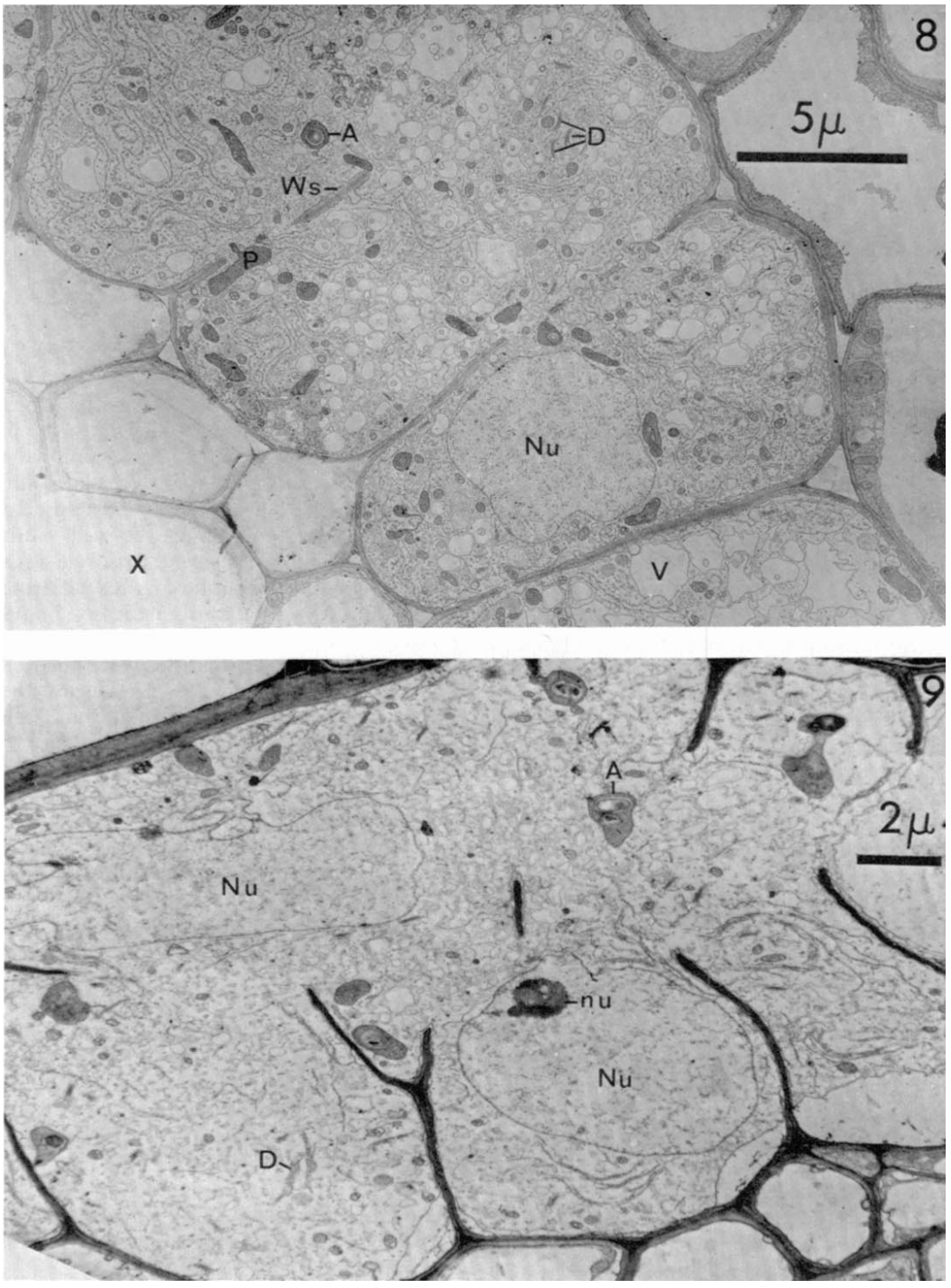


FIG. 8-9. **8)** 'Lee' soybean four days after inoculation showing the early predominantly anabolic stage of syncytial development with enlarged cells filled with cytoplasm and thickening cell walls and wall segments. Note numerous plastids, amyloplasts, dictyosomes, small vacuoles, rough endoplasmic reticulum and an enlarged nucleus. **9)** 'Lee' soybean nine days after inoculation showing multinucleate condition formed by cell wall lysis and separation. Nuclei are enlarged and more irregular in shape when using the following modification in technique; specimen grown in agar, alcohol dehydrated, Maraglas-embedded, and viewed with a Philips 300 EM at 40 KV. A = Amyloplast, D = dictyosome, Nu = nucleus, nu = nucleolus, P = plastid, V = small vacuole, Ws = wall segment, X = primary xylem.

formations were evident, and many of these contained vesicles, microfibrils, and possibly parts of the golgi apparatus (Fig. 15, 16). Dense coalesced cytoplasm, containing numerous small vacuoles and organelles, filled most of the cells forming the syncytia. The plasmalemma appeared continuous throughout the syncytia and surrounded wall fragments. Numerous large plastids and amyloplasts were present. Considerable increase in numbers of ribosomes, rough endoplasmic reticulum, dictyosomes, and mitochondria were noted. Enlarged nuclei and nucleoli were noted. By day 9, syncytia had formed through the coalescing of cytoplasmic contents of adjacent cells (Fig. 9). The coalescence of cytoplasmic components between cells and syncytial development were increasingly evident the closer the infected cells were to the prosyncyte. By the 14-16th day following infection mitochondria were numerous and exhibited a variety of shapes as did the plastids (Fig. 10, 11, 12, 13). Dictyosomes were less numerous than in the 4-9 day infections. The endoplasmic reticulum ranged from a few scattered to several parallel strands (Fig. 10, 11). The number of boundary formations also varied from very few to many per cell. Some small inconspicuous protuberances occurred on thickening cell walls near the infection site (Fig. 12, 13) while the walls of hypertrophied cells near the periphery of the infection were relatively smooth, evenly layered, and about five times thicker than normal (Fig. 10, 11, 14). Some boundary formations appeared to contain microfibrils which were being deposited on the secondary wall. These microfibrils seemed to be formed from the electron-dense outer layers of the microvesicles (Fig. 14). It was often noted that syncytial cell walls were thin and secondary wall deposits absent or greatly reduced where plasmodesmata occurred (Fig. 10). Breakdown and collapse of several endodermal cells near the infection site was common. Some of the outermost primary xylem vessels and inner most cortical cells near the center of the infection were slightly affected and crushed by the enlarging syncytium. Many irregular, cup-shaped, and possibly doughnut-shaped mitochondria were evident (Fig. 10-13). The nucleus appeared enlarged and usually retained a spherical shape. Plastids containing many electron dense spherical inclusions were observed (Fig. 10, 11). Boundaries of small vacuoles often appeared very electron dense. There were sharp demarcations between syncytial and relatively normal appearing pericycle cells at the periphery of the syncytium even though the cells were connected by plasmodesmata (Fig. 10). Cells largely or entirely filled with an amorphous material associated with the cell walls were seen often (Fig. 17). Tissues examined from 21-day-infections appeared essentially the same as in 14- to 16-day infections, and were not included here.

Resistant reaction: The rate of development of the resistant reaction varied more than that of the susceptible reaction. The initial stages of infection appeared similar to those in susceptible plants. When observed, the nematode stylet was inserted into an endodermal cell, not necessarily located near the protoxylem pole, and the cell walls between it and the adjacent pericycle cells were partly lysed and ruptured, thus initiating a syncytium. Lysed cell walls in the first 2 days of infection appeared more irregular, and the observed boundary formations were not well organized (Fig. 18, 19, 20, 21). During the next 2 days, much of the cell wall and cell contents were lysed (Fig. 22, 23). Collapse of endodermal cells was observed often and lysis of xylem walls was evident. However, various stages of cell wall lysis and cytoplasmic disorganization could be observed at later time periods (Fig. 24, 25, 26). Cells that were still lysing 2-4 days after infection had very few recognizable organelles. No evidence of a shift to an anabolic metabolism with the accompanying cell wall regeneration, boundary formation, or secondary wall deposition was noted. Two resistant reactions in Peking roots were noted: (i) the most common appeared as a large darkened necrotic syncytial cavity composed of lysed cell walls and cytoplasm (Fig. 22, 23); and (ii) a less-frequent more localized reaction involving only a few collapsed or partially collapsed cells which were filled with an amorphous material suggesting localization of lysis.

DISCUSSION

In this discussion the term syncytium, when referring to *R. reniformis* infection,will pertain to a multinucleated condition which

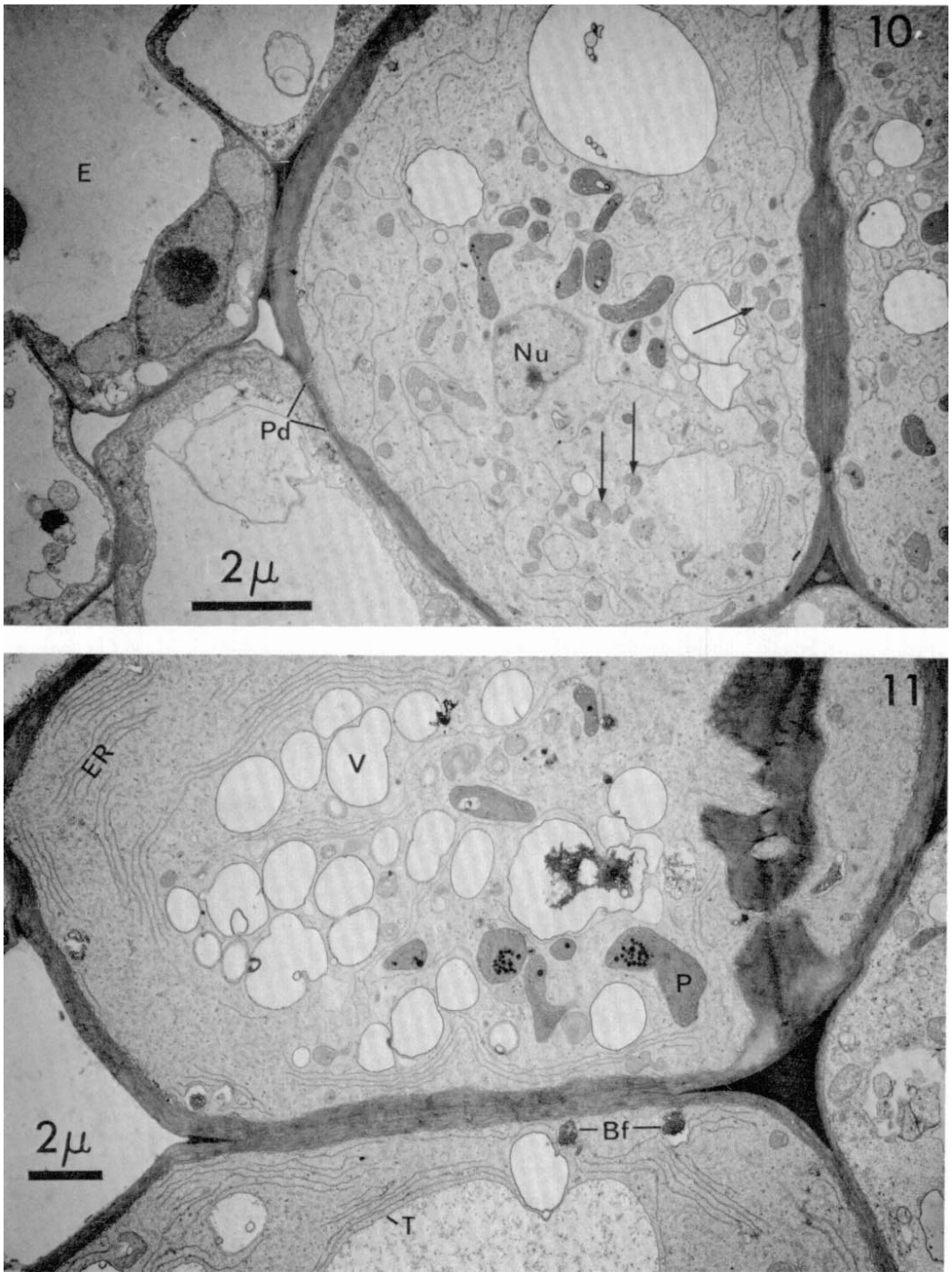


FIG. 10-11. 'Lee' soybean 16 days after inoculation. **10)** Shows a peripheral giant cell (center) connected by plasmodesmata to a relatively unmodified pericycle cell (lower left). Note large irregularly shaped plastids and numerous irregular or cup-shaped mitochondria (arrow). Secondary wall deposits are smooth, dictyosomes and boundary formations are few and only a few scattered strands of endoplasmic reticulum are present. **11)** Hypertrophied cells near the periphery containing several long parallel strands of endoplasmic reticulum, large irregular shaped plastids and mitochondria, and a few dictyosomes. A large vacuole and several small vacuoles are also present. Bf = boundary formation, E = endodermis, ER = endoplasmic reticulum, Nu = nucleus, Pd = plasmodesmata, T = tonoplast, V = vacuole.

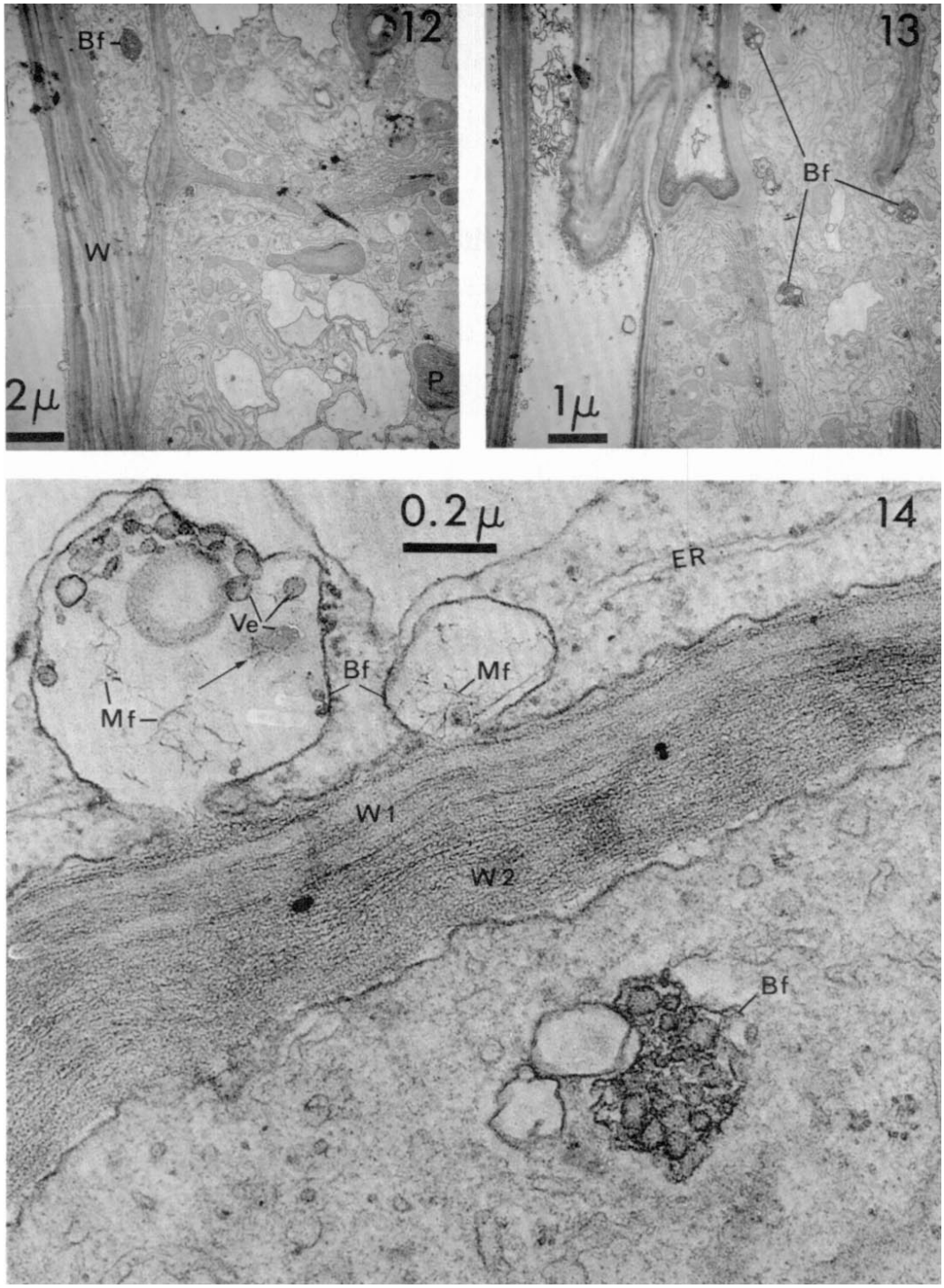
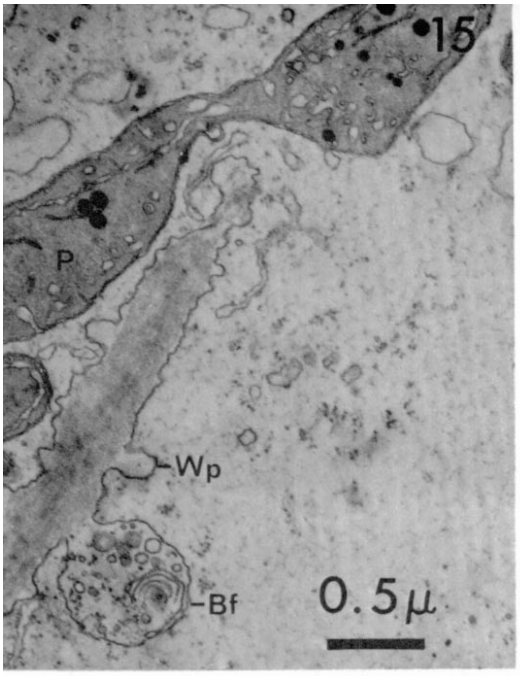
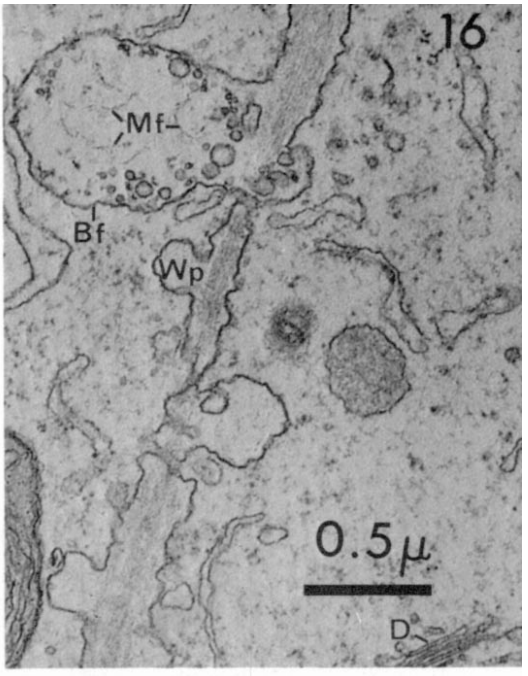
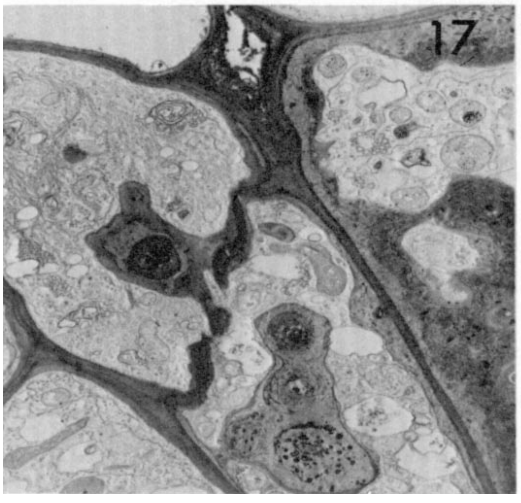


FIG. 12-14. 'Lee' soybean 16 days after inoculation. **12-13)** Longitudinal section showing vestigial walls and wall breaks occurring in longitudinal and transverse cell walls of the syncytium. Boundary formations occur on most walls. Cytoplasmic organization near the center of the syncytium is apparent. **14)** Enlargement of hypertrophied cell wall near the periphery of a syncytium exhibits smooth secondary wall deposits. Boundary formations containing numerous vesicles and some microfibrils which appear to be contributing to the formation of secondary wall. Microfibrils appear to be associated with the dense outer layers of some of the vesicles (arrow). Bf = boundary formation, ER = endoplasmic reticulum, Mf = microfibril, Ve = vesicles, W_1 = primary wall, W_2 = secondary wall.







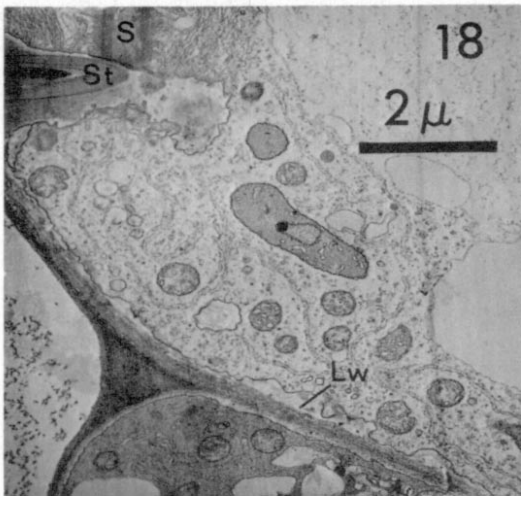
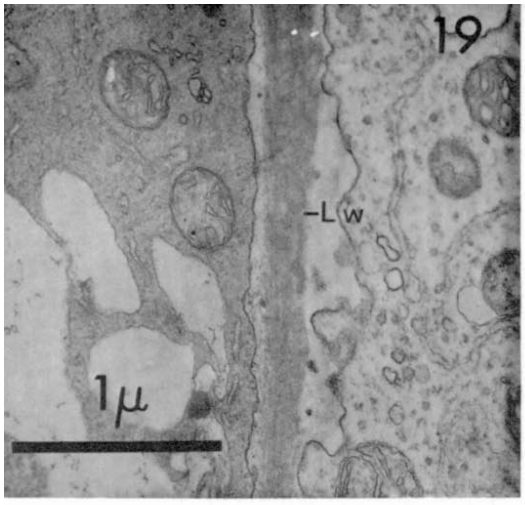


FIG. 15-18. **15-16)** 'Lee' soybean four days after inoculation showing the early anabolic stage of syncytial wall development. Boundary formations containing many vesicles, layered cisternae-like membranes, and microfibrils were seen. Small wall protuberances near the centers of the syncytia were common at this time. **17)** 'Lee' soybean 18 days after inoculation showing amorphous masses of wall material containing inclusions and occupying large portions of the protoplast. Specimen prepared as per Fig. 9. **18)** 'Peking' soybean two days after inoculation showing nematode stylet inserted in the prosyncyte and wall lysis in progress. Secretion from the nematode stylet was also noted. Bf = boundary formation, Lw = lysing wall, P = plastid, S = nematode secretion or exudate, St = stylet, Wp = wall protuberance.


19
Lw
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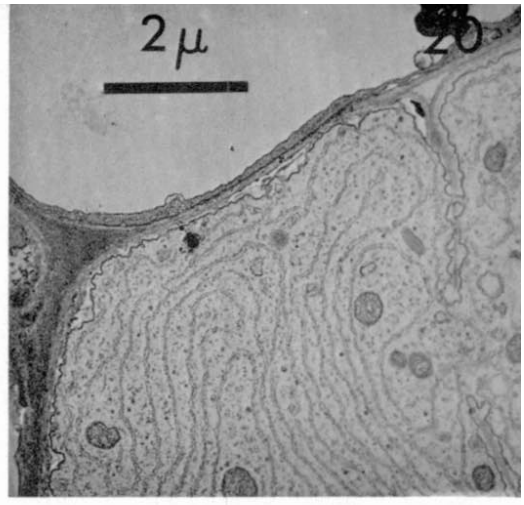
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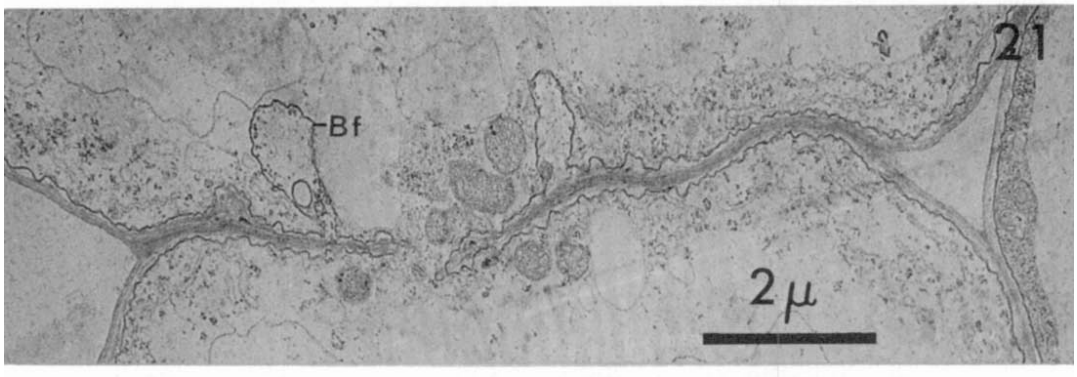
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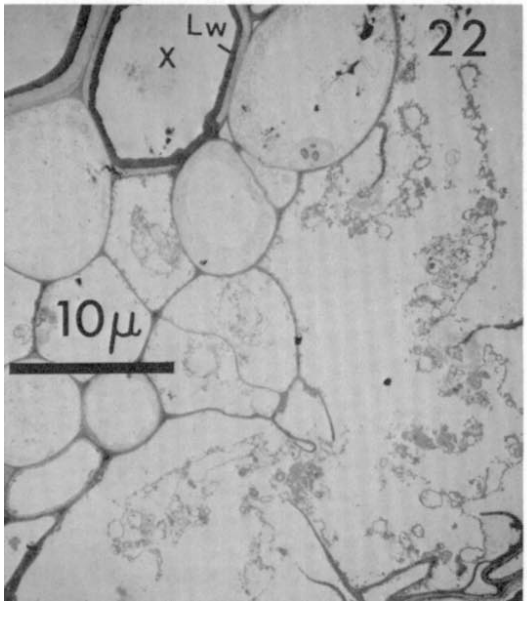
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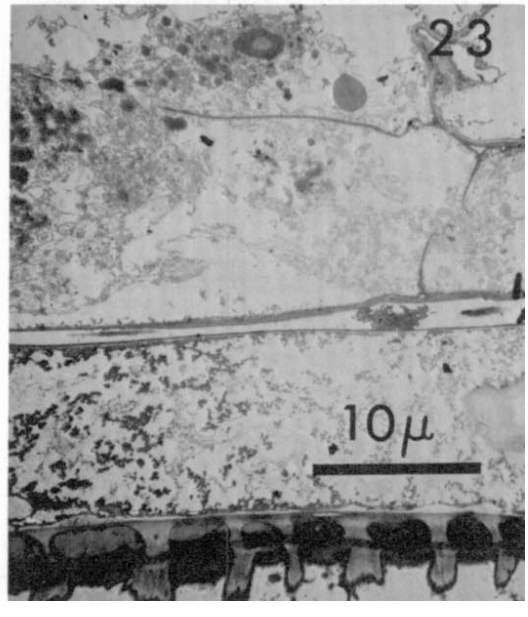
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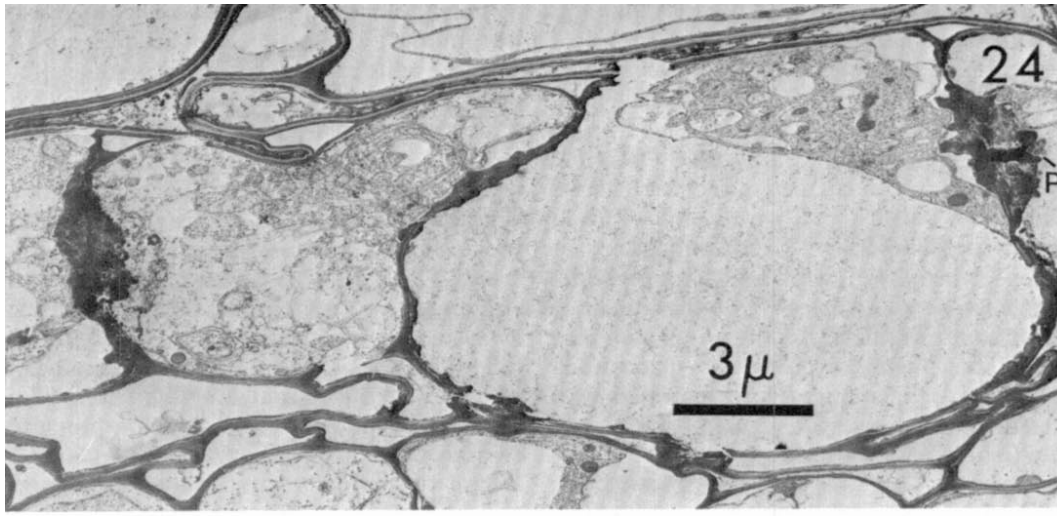
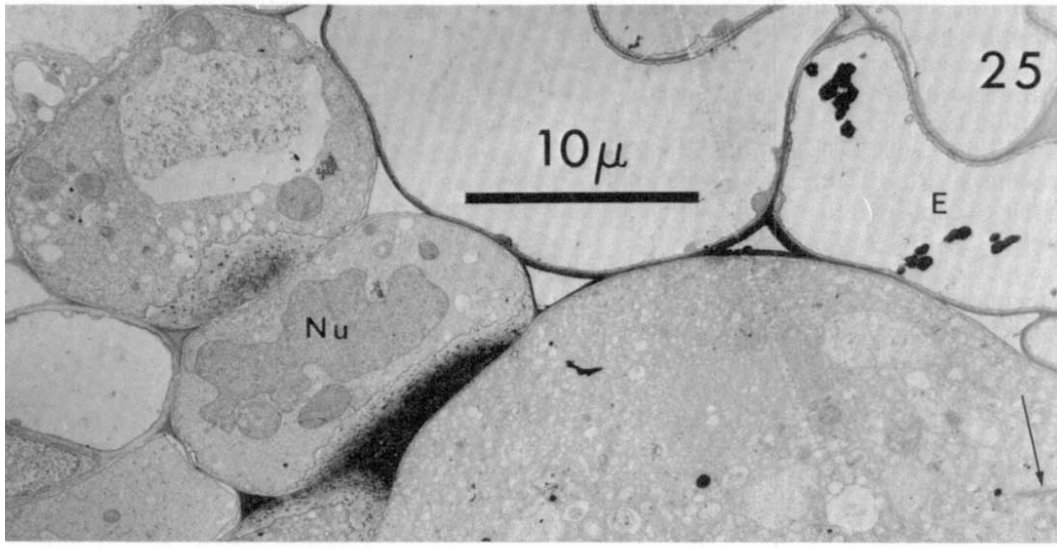
10μ

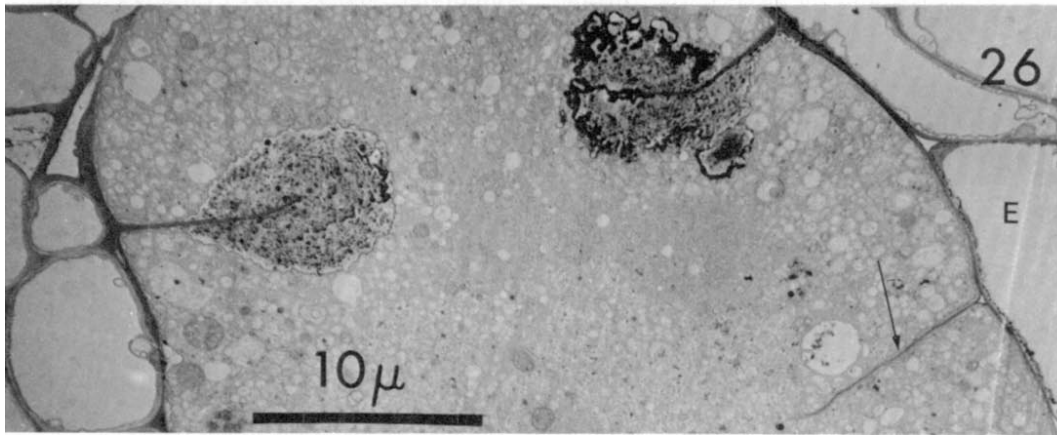
resulted from a partial dissolution and separation of cell walls and a coalescence of cytoplasmic components. It will also include peripheral hypertrophied cells induced by *R. reniformis* infection where cell walls appear relatively contiguous, preserving the original uninucleate condition. *R. reniformis* penetrated cortical cells intracellularly and usually perpendicular to the central axis of young soybean roots which confirms previous light microscopic work (28). Observations of the alterations in cell walls suggested that cortical penetration was basically mechanical rather than enzymatic. The penetrated wall pressures on the sessile female's body may help to hold her position and/or prevent entry of some other foreign organisms or substances. Upon reaching the inner cortical region, the infective female became sedentary and only the stylet was inserted in the initial cell of the syncytium. On five occasions where the initial cell of the developing syncytium was identifiable it was endodermal. However, the possibility of a pericycle or other cell being the prosyncyte is not ruled out; but we were unable to confirm it by positive cell and tissue identification. Identification of the prosyncyte, the initial cell of the developing syncytium, may be very important to the understanding of some types of resistant and susceptible phenomena for the following reasons: (i) through indirect comparisons it has been shown on susceptible Lee that female *R. reniformis* (28) and *H. glycines* (7, 8) generally developed better near protoxylem poles than near protophloem poles; (ii) newly formed syncytia were made primarily of pericycle tissues and the prosyncyte; (iii) the chemistry, function and development of endodermal cells near the protoxylem pole can vary considerably from that at the protophloem region (32). For these reasons, it is suggested that the chemistry of the endodermis and/or nematode orientation on gap (32) or passage cells (11), which are usually associated with the protoxylem pole, may be important to favorable syncytium and nematode development. Consequently one of the important questions to be answered is whether or not the prosyncyte has some effect on the susceptibility or resistance to one or more strains or species of nematodes.

Cell wall lysis and/or breakdown, which occurs between adjoining cells and initiates syncytial formation is consistent with most previous reports on sessile nematodes (4, 6, 7, 9, 12, 17, 22, 24, 25, 26, 33). The apparent stretching of cell walls through internal cytoplasmic pressures and/or a weakening of the wall through partial lysis seems important in syncytial formation and hypertrophy of cells. Presumably the internal pressures exerted on intact cell walls of syncytia could be greatly increased by nematode secretions and increased osmotic pressures resulting from the accumulation of smaller more soluble molecules derived from the lysis of larger relatively insoluble molecules and/or the synthesis of soluble molecules. The combination of cell wall lysis and internal cytoplasmic pressure eventually causes the parting of several cell walls near the center of the syncytium resulting in cytoplasmic coalescence. Consistent with reports on *H. glycines*-induced syncytia on Lee and Peking (7, 8, 13, 29), endomitosis without cytokinesis does not appear to be an important factor in *R. reniformis* syncytial formation as found in some root-knot nematode infected tissues (15). During the early or lysis stage of syncytial development the tonoplasts surrounding the large central vacuoles broke down, or were rearranged in some way, and the cells became filled with a dense cytoplasm containing many small vacuoles. This is consistent with *H. glycines* infection on soybean (7, 13). Some changes in cell organelles were also noted and the results are relatively consistent with most other reports of syncytia induced by sedentary nematodes. Boundary formations and paramural bodies were scarce, degenerate, or absent from lysing walls. The nature and precise source of the heterogeneous-appearing secretion, proximal

FIG. 19-23. **19-21)** 'Peking' soybean two days after inoculation. **19)** Lysing wall. **20)** Lysing walls with cell organelles relatively intact and numerous cisternae of rough endoplasmic reticulum. **21)** Shows relatively advanced stage of cell wall and cytoplasmic lysis. Note relatively poor condition of remaining boundary formations and cell organelles. **22-23)** 'Peking' soybean four days after inoculation showing the hypersensitive reaction. **22)** Advanced stages of lysis involving many types of cells shows pericycle and xylem parenchyma cell damage accompanied by hydrolysis of primary xylem at upper left and collapse of endodermal tissue at bottom right corner. **23)** Longitudinal section showing advanced syncytial lysis. Bf = boundary formation, Lw = lysing wall, X = xylem.


24
3μ


25
10μ
E
Nu


26
E
10μ

spherical bodies, and unidentified bodies (Fig. 1, 2, 3) were not determined. It is realized that the excretion shown could have been induced during the killing and fixing process and may have come from material contained in the gut, the esophageal glands, or both. The proximal spherical and unidentified bodies may have come from some interaction between nematode secretions and cytoplasmic components. However, our in situ observations tend to support Bird's (3) in vitro observations that stylet exudates can consist primarily of particles of much the same dimensions as ribosomes. The dense spherical bodies are also made up of ribosome-size particles and their appearance at the interface between the observed secretion and host cytoplasm may have some relationship to dorsal esophageal granules found at the periphery of stylet exudates of *Meloidogyne javanica* (3). Some of the dense spherical bodies appear to separate into smaller components in the cytoplasm of the host (Fig. 3), Epstein and Cohn have suggested that RNA may be injected into roots by feeding *Longidorus africanus* (10). The characterization of particular nematode secretions also has to be one of the prime considerations for understanding the basis for host-parasite interactions. If RNA is secreted by phytoparasitic nematodes, the protein(s) programmed for may not be isolated directly from the nematode.

Early in syncytial formation, partially lysed swollen portions of the cell walls stained heavily and heterogeneously blue to purple with toluidine blue while the unlysed vestigial parts of the primary wall were green. The green color appeared to diffuse particularly where cell wall separation was in progress or about to take place. This suggests that phenolic compounds and/or perhaps lignin components were being released from the primary cell walls through lysis which supports observations of *H. rostochiensis* parasitism of potato (17). Partial wall dissolution was observed at the prosyncyte, endodermal cells adjacent to the prosyncyte, and in all syncytial walls, but it was most evident between pericycle cells.

The number of pericycle cells to be incorporated in a syncytium appeared to be largely determined within the first 4-9 days of inoculation. In heavily parasitized young roots all the pericycle cells found in a fixed cross section sometimes appeared affected. However, a syncytium incited by one female usually extended laterally in both directions from the protoxylem pole near the infection sight in a young tetrarch root, through the pericycle, to within one or two pericycle cells of the adjacent lateral protoxylem poles. The lateral boundary between syncytial and relatively normal cells was sharp, even though the cells were connected through plasmodesmata (Fig. 10). This suggests that the number of pericycle cells (usually between 100-200) incorporated into a single syncytium was determined mostly during the partial wall lysis phase of development; and/or that some of the primary agents responsible for syncytial development may be too large to pass easily or unassisted through plasmodesmata.

During the first two days of infection, syncytia in both Lee and Peking appeared to have nearly parallel development, although the rate of wall lysis proceeded faster in Peking. Sometime between the second and fourth day following soil infestation, syncytia in Lee passed from the partial wall lysis stage to an anabolic stage of development. This transition was marked by the almost complete interruption of wall lysis accompanied by secondary wall thickening of all syncytial cell walls, including the vestigial and fragmented ones. Boundary formations in syncytia induced by nematodes were first reported by Huang and Maggenti (16) and subsequently by others (9, 13, 17, 22, 29). Boundary formations of all three types described by

FIG. 24-26. **24)** 'Peking' soybean syncytium nine days after inoculation showing enlarged or thickened pericycle walls apparently in an incomplete or arrested state of lysis. Note very dark area at plasmodesmata. From the disorganized state of the cytoplasm and walls, it does not appear that thickened walls were created by the deposition of wall materials. Specimen prepared as in Figure 9. **25-26)** 'Peking' soybean 18 days after inoculation showing progressive stages of cell wall dissolution. **25)** Early lysis upper left wall to very advanced separated wall lower right (arrow). Middle lamella and outer endodermal cell walls nearest the syncytium are very electron dense suggesting some lysis is taking place there also. Giant cell contains an enlarged convoluted nucleus. **26)** Parted cell wall with lysed wall materials still very evident, plus a vestigial wall (arrow). Compare with wall breakdown of 'Lee' soybean in Fig. 6-7. Note that few organelles are associated with cytoplasm in the syncytial area as compared to 'Lee'. After 14-16 days of infection and retarded lysis, there is no evidence of secondary wall deposits. E = endodermal cell, Nu = nucleus, Pd = possibly a distorted plasmodesmata.

Esau were noted in Lee syncytia, but types with vesicular bodies were predominant and associated with orderly secondary wall deposits following the early lysis stage of infection. No microtubules were seen associated with boundary formation in Lee or Peking which agrees with the observations of Gipson et al (13) on Lee, but differs from observation by Riggs et al (29) on Peking. However, variations in techniques, fixation process, or the parasites may account for the differences. Sessile *R. reniformis* act as a nutrient sink with the syncytia acting as the nutrient source. No transfer cells with large wall protuberances were found in this study as reported in *H. rostochiensis*-induced syncytia in potato roots (17).

In the 16- to 21-day syncytial development there was considerable crushing of adjacent primary xylem vessels at the protoxylem pole as observed with *H. glycines* on Lee (13).

Two basic, probably related, types of hypersensitive reactions were noted. At various intervals of time we observed thickened cell walls in Peking. These walls had varying electron densities and the most dense areas were oriented along what appeared to be originally plasmodesmata. Blockage of plasmodesmata in *H. glycines* infected Peking was reported by Riggs et al. (29). They also noted considerable boundary formation and microtubules associated with cell wall thickening which was interpreted as a possible walling off of the parasite in Peking. In a preliminary report on disease resistance (26), Peking syncytia walls appeared thicker than in Lee and may possibly contribute to a walling off of the parasites feeding site (Fig. 24). We know that a small percentage of female *R. reniformis* can mature and lay viable eggs (Rebois, et al., *unpublished*) on resistant Peking. Thus it was probable that this cultivar can provide suitable syncytia for nematode development. However, the major process in this type of resistance seems to be relatively uncontrolled lysis. Thickened cell walls in Peking syncytia resulting from *R. reniformis* parasitism appears to result from an arrested or suspended state of lysis rather than secondary wall deposits, because the cytoplasm generally lacks the organization and organelles for extensive anabolic regeneration. Ultimately the hypersensitive reaction denies the parasite a suitable energy source for development. While *R. reniformis* on Peking are less osmophilic than on Lee, indicating death by starvation, death by toxins has not been ruled out.

Results of this study indicated that, in addition to more accurately characterizing nematode excretions, future research on extending the localized lysis phase and/or preventing the anabolic phase in nematode-induced syncytia may provide some practical means of inducing nematode resistance in otherwise susceptible cultivars.

Finally, most light-microscopic histopathological reports on *R. reniformis* parasitism of other host plants have not reported any coalescence of cytoplasmic components between hypertrophied or giant cells, and this may be the case. However, we observed that most of the holes or wall separations between hypertrophied pericycle cells in soybeans were less than 15 μm, and would not be readily seen in thick sections.

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