Ultrastructural Changes in Peking Soybeans Infected With Heterodera glycines

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ABSTRACT

Syncytia were observed in Peking soybean within 42 hr after inoculation with larvae of *Heterodera glycines*. Fine structural observations of syncytia revealed cell wall perforations and prominent cytoplasm containing numerous plastids, and mitochondria and proliferated endoplasmic reticulum. Syncytia were beginning to degenerate, and cell wall thickenings were prominent 4 days after inoculation. Seven days after inoculation, cytoplasmic organelles were no longer distinguishable. In addition, large segments of cell wall protruded into the syncytium and the surrounding cells. Lipid globules were frequently observed in the degenerating syncytia. The deposition of secondary wall material, which seals off a diseased area, may be the major mechanism of resistance against *H. glycines*.

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The soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952, stimulates syncytium development in susceptible cultivars of soybean. Ultrastructural development of syncytia in roots of susceptible Lee soybean has been reported (4). Syncytium development in the roots of soybean and other plants infected by cyst or root knot nematodes was also reviewed in a previous publication (4).

Morphological changes in Peking soybean, a cultivar resistant to reproduction of *H. glycines*, were

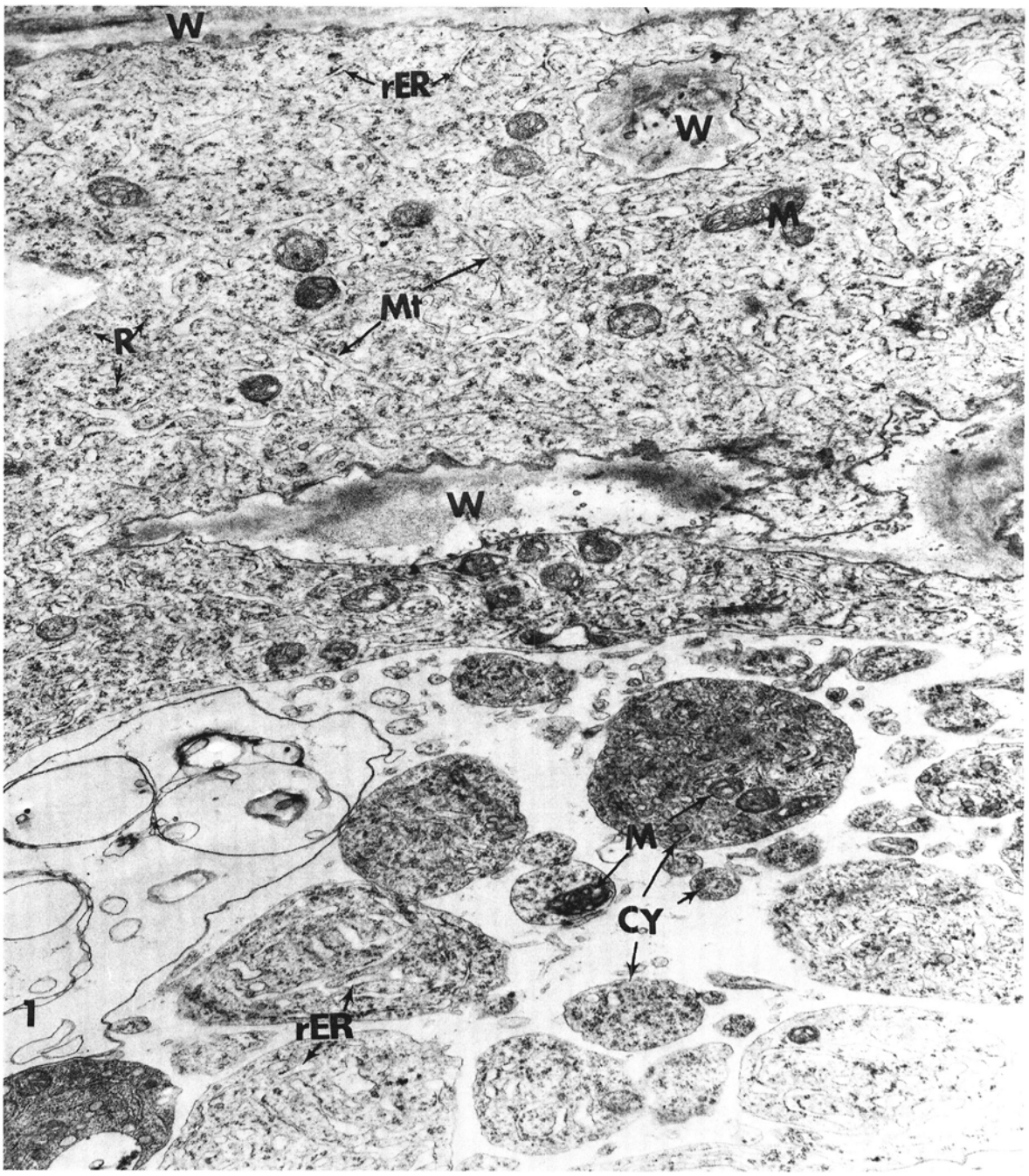


Fig. 1. A portion of a syncytium 42 hr after inoculation. Numerous microtubules, rough endoplasmic reticulum, and clusters of free ribosomes can be observed in the cytoplasm. In addition, cell wall perforations in the cytoplasm and cytoplasmic protrusions in the vacuole are commonly present (× 15,000). CY = cytoplasm; M = mitochondrion; Mt = microtubule; rER = rough endoplasmic reticulum; W = cell wall.

previously studied with light microscopy by Ross (12) and Endo (2). Ross reported necrosis and cellular disorganization around the anterior end of nematode larvae, but did not observe syncytia. Endo described syncytium formation 2-3 days after inoculation. He observed cellular changes similar to early reactions in susceptible soybean cultivars. Within 5 days after inoculation, syncytia degenerated and necrotic tissue gradually infiltrated the syncytial area.

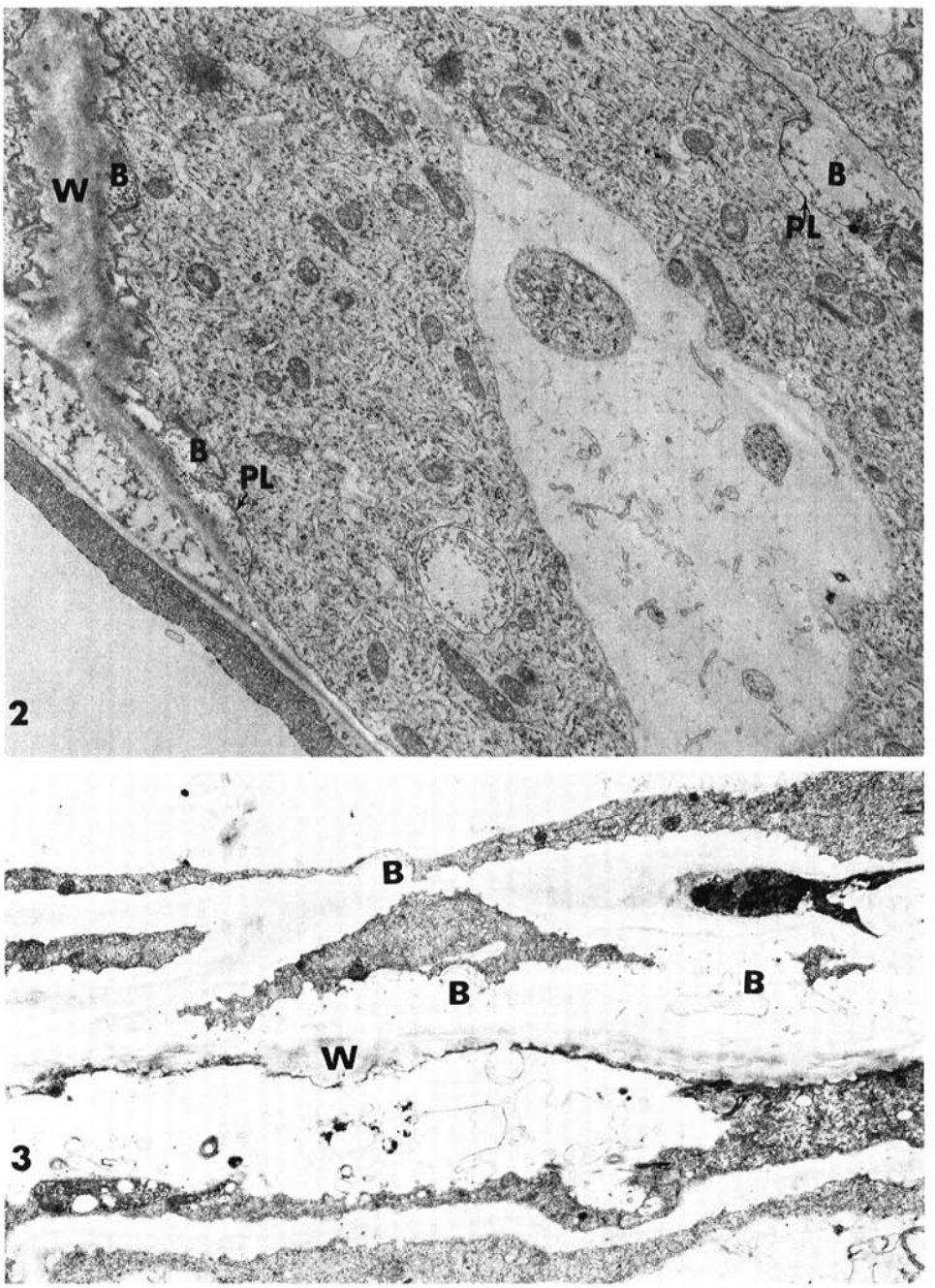


Fig. 2-3. 2) Irregular thickening of cell wall in a portion of a syncytium 42 hr after inoculation. The plasmalemma is frequently invaginated into the cytoplasm, forming pocketlike boundary formations which contain membranous vesicles and tubules. (× 12,000). **3)** Portions of syncytium-component cells showing thickened cell walls 4 days after inoculation. Boundary formations are directly associated with cell wall thickenings (× 6,000). B = boundary formation; PL = plasmalemma; W = cell wall.

This ultrastructural study documents the progressive changes in Peking soybean root cells infected by *H. glycines* after inoculation.

MATERIALS AND METHODS.–Seeds of soybean [*Glycine max* (L.) Merr. 'Peking'] were planted in white quartz sand and grown in the greenhouse at 24-30 C. Seven days later, germinated seedlings at the cotyledonary stage were inoculated with eggs and larvae of *Heterodera glycines* (10). The roots were exposed to larval penetration for 24 hr; then the seedlings were transplanted into fresh sterile sand. Forty-two hr, 4 days, and 7 days after inoculation, root samples were washed thoroughly, fixed in glutaraldehyde, and postfixed in 1% osmium tetroxide. Nematodes were visible in the roots stained with osmium. Small, excised root sections containing nematodes were stained 8-16 hr in 0.5% uranyl acetate (1), dehydrated, and embedded in Epon 812 (8). Noninoculated controls were prepared similarly. Sections, 1 μ thick, were stained with toluidine blue (14) and viewed with a light microscope to verify the presence of nematodes and syncytia. When a syncytium was present, 800- to 900-Å sections were stained with 2% uranyl acetate followed by lead citrate (9), and examined with a Siemens Elmiskop 1A.

RESULTS.–Noninoculated Peking soybean roots appeared similar to those previously described for noninoculated Lee soybean roots (4).

Second stage larvae of *H. glycines* entered Peking soybean roots, migrated through the cortex, and penetrated cells with their stylets. Syncytia were initiated within 42 hr after inoculation. Cytoplasm in the syncytia contained numerous plastids, mitochondria, and a greatly proliferated amount of endoplasmic reticulum (ER). Numerous ribosomes were either bound to the membranes of the ER or clustered in the ground cytoplasm (Fig. 1). Microtubules were numerous, and were distributed randomly in the cytoplasm of syncytium-component cells (Fig. 1). In some cells, areas of prominent cytoplasm protruded into the central vacuole, resulting in numerous circular masses of cytoplasm surrounded by the tonoplast (Fig. 1). These circular masses contain ER, ribosomes, and mitochondria.

Linearly aligned cell wall fragments in the cytoplasm of syncytium-component cells were evidence of cell wall perforations. These often appeared as isolated wall fragments (Fig. 1). Some syncytia exhibited an irregular thickening of the inner wall, and the plasmalemma was invaginated into the cytoplasm forming pockets containing small membranous vesicles or tubules (Fig. 2) which appeared to be structurally similar to the "boundary formations" of Esau et al. (3) and "paramural bodies" of Marchant & Robards (6) and Robards (11).

Some cells around the nematode were necrotic, apparently as a result of mechanical damage during nematode penetration, but these were not involved in the formation of the syncytium. No necrosis was observed in syncytium-component cells at this stage of infection.

Four days after inoculation, perforations of the cell wall in syncytium-component cells were more pronounced than those at 42 hr. The wall thickenings of syncytia were more pronounced, and were very irregular along the original cell wall (Fig. 3). Boundary formations were more numerous, and often were directly associated with the thickened areas of the cell walls (Fig. 3). The thickened cell wall areas contained numerous electron-dense materials (Fig. 3).

Four days after inoculation, syncytia were undergoing degeneration (Fig. 4). In the degenerating cells, the cytoplasm contained numerous vacuoles of various sizes and irregular aggregations of the ER membrane (Fig. 4). Masses of cytoplasm protruding into the central vacuole appeared more degenerated than the parietal cytoplasm. The cytoplasmic matrix of syncytia contained a random distribution of membrane remnants, membrane aggregates, and electron-dense particles (Fig. 4). During the early stages of degeneration, many osmiophilic, lipidlike globules of various sizes were randomly distributed in the cytoplasm (Fig. 4, 5).

Cells surrounding nematodes and syncytia exhibited severe mechanical damage. Cell wall breakage and cytoplasmic and nuclear disintegration could also be observed.

The nematodes were adjacent to the syncytia (Fig. 6). Occasionally, the stylet of the nematode was observed penetrating the cell wall of the syncytium (Fig. 6). The cytoplasm of this cell exhibited characteristics typical of syncytia (4) such as cell wall perforations, increased number of mitochondria, and ER.

Syncytia were very degenerated 7 days after inoculation. In these syncytia, cell organelles such as chloroplasts and mitochondria were no longer distinguishable (Fig. 7). The cytoplasm was extremely electron-dense and clumped. Cytoplasmic degeneration was also apparent in one or two layers of cells surrounding the syncytia (Fig. 7).

After 7 days, cell wall thickenings occurred in syncytia and in some cells adjacent to syncytia which showed cytoplasmic degeneration (Fig. 7). The extreme degree of wall thickening was characterized by protrusions of wall material into the cytoplasm of syncytia and adjacent cells (Fig. 7). These bulbous protrusions of cell wall were not observed before 7 days after inoculation. The cells containing cell wall thickenings were usually necrotic. All thickened areas contained electron-dense, granular materials (Fig. 7). In distal portions of the wall thickenings, aggregations of microtubules were observed in the cytoplasm adjacent to the thickened wall areas (Fig. 7). Plasmodesmata were not observed in the thickened wall areas.

Some nematodes appeared to have been blocked from feeding in the syncytium by the extremely thickened cell wall (Fig. 8). Nonnecrotic cells adjacent to syncytia showed increased dimension of the cytoplasm with proliferated ER and ground cytoplasm.

DISCUSSION.–This study confirms the report by Endo (2) which showed that syncytia are formed in resistant Peking soybean invaded by *H. glycines* and

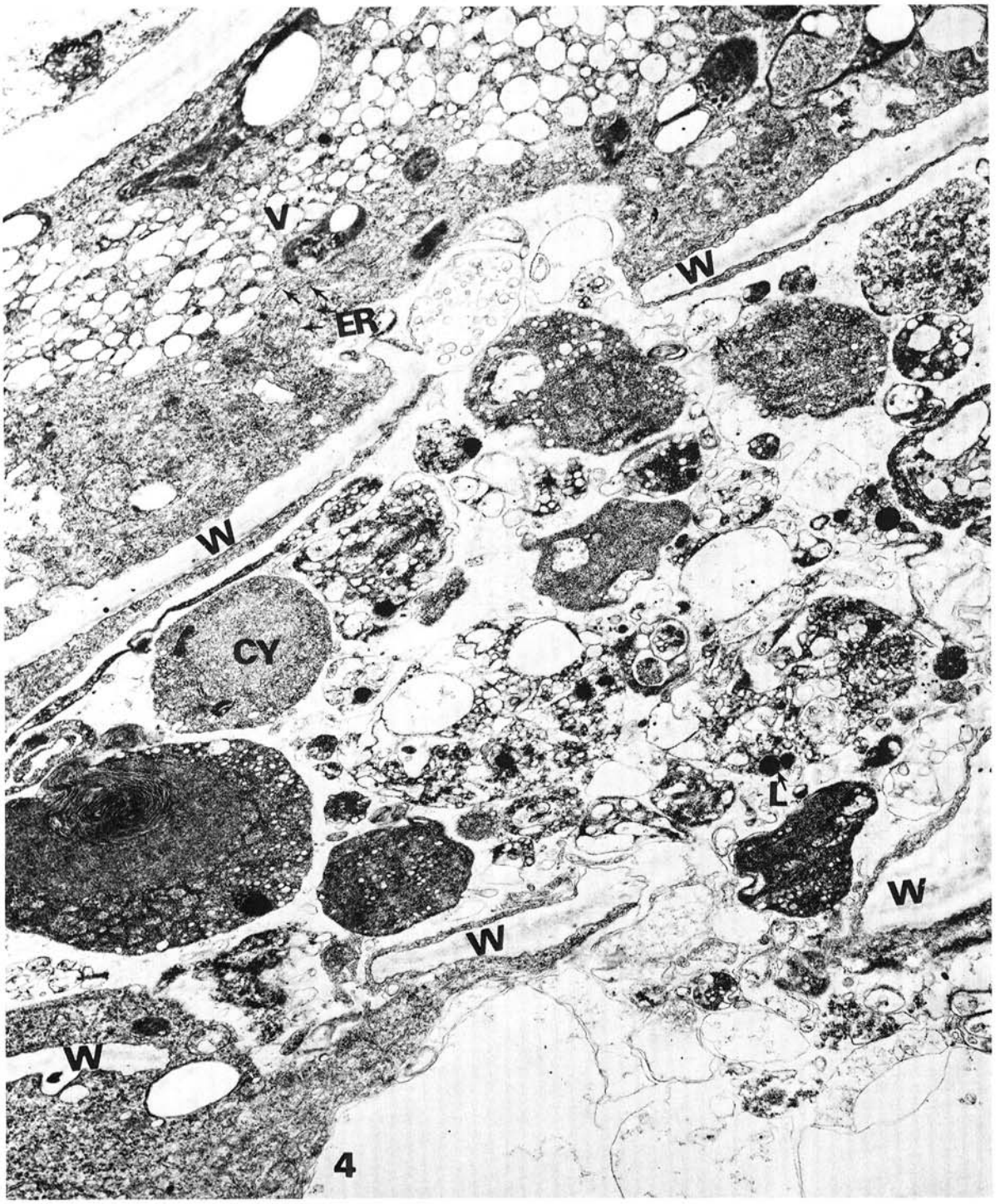
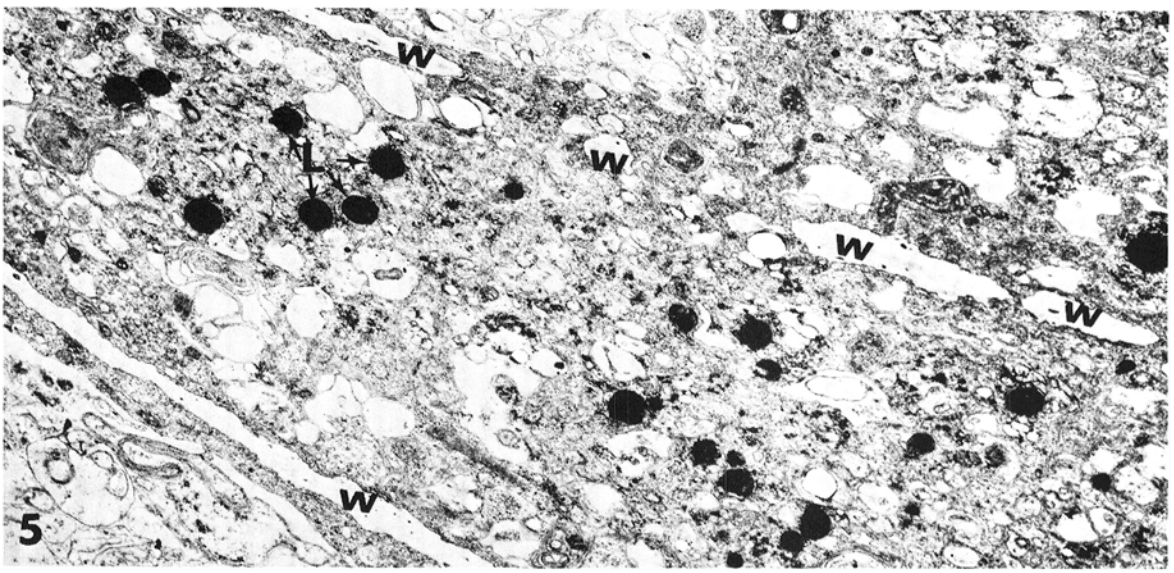


Fig. 4. Portion of degenerating syncytium 4 days after inoculation. At this time, the cytoplasm becomes vacuolate, and appears electron dense. Membrane remnant, dense particles, and segments of cell walls are present (× 12,000). CY = cytoplasm; L = lipid globule; V = vacuole; W = cell wall.



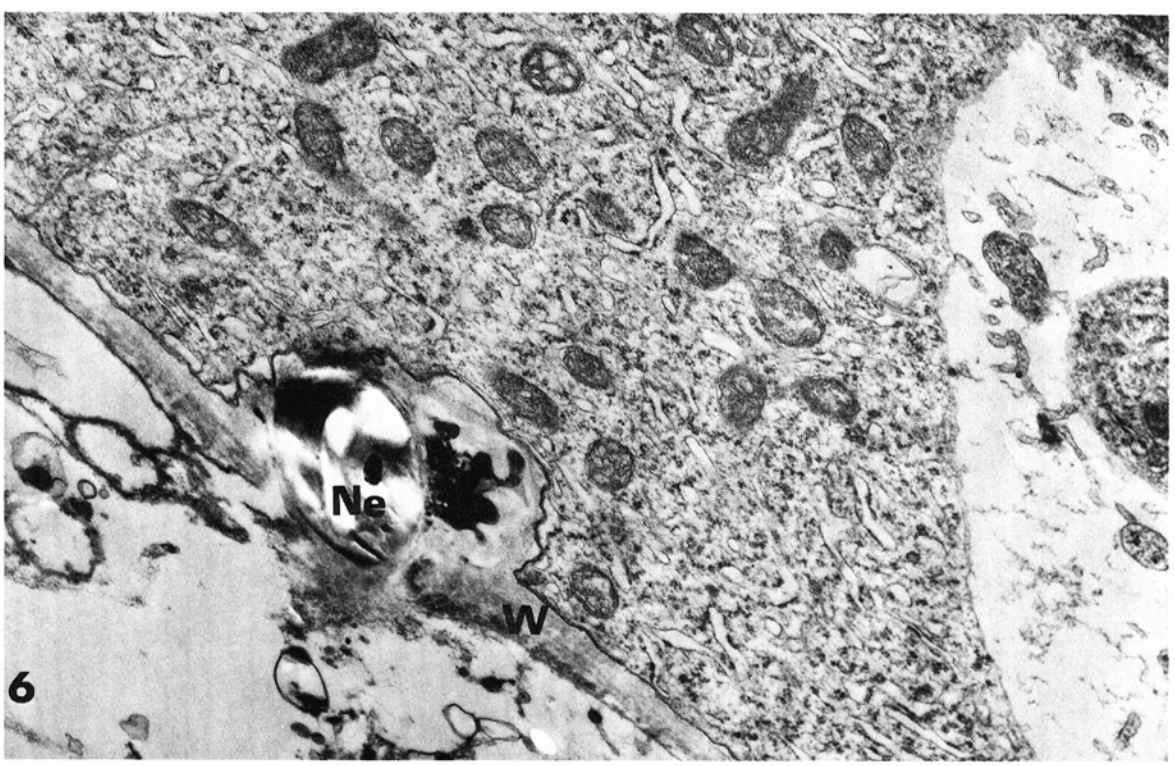


Fig. 5-6. 5) Syncytium 4 days after inoculation with numerous, lipidlike globules of various sizes scattered in the cytoplasm. Vacuolation of cytoplasm and loose membrane aggregates indicate cell degeneration (× 6,000). **6)** Portion of a syncytium 4 days after inoculation. Cell wall has been penetrated by a nematode stylet (× 18,000). Ne = nematode; W = cell wall.

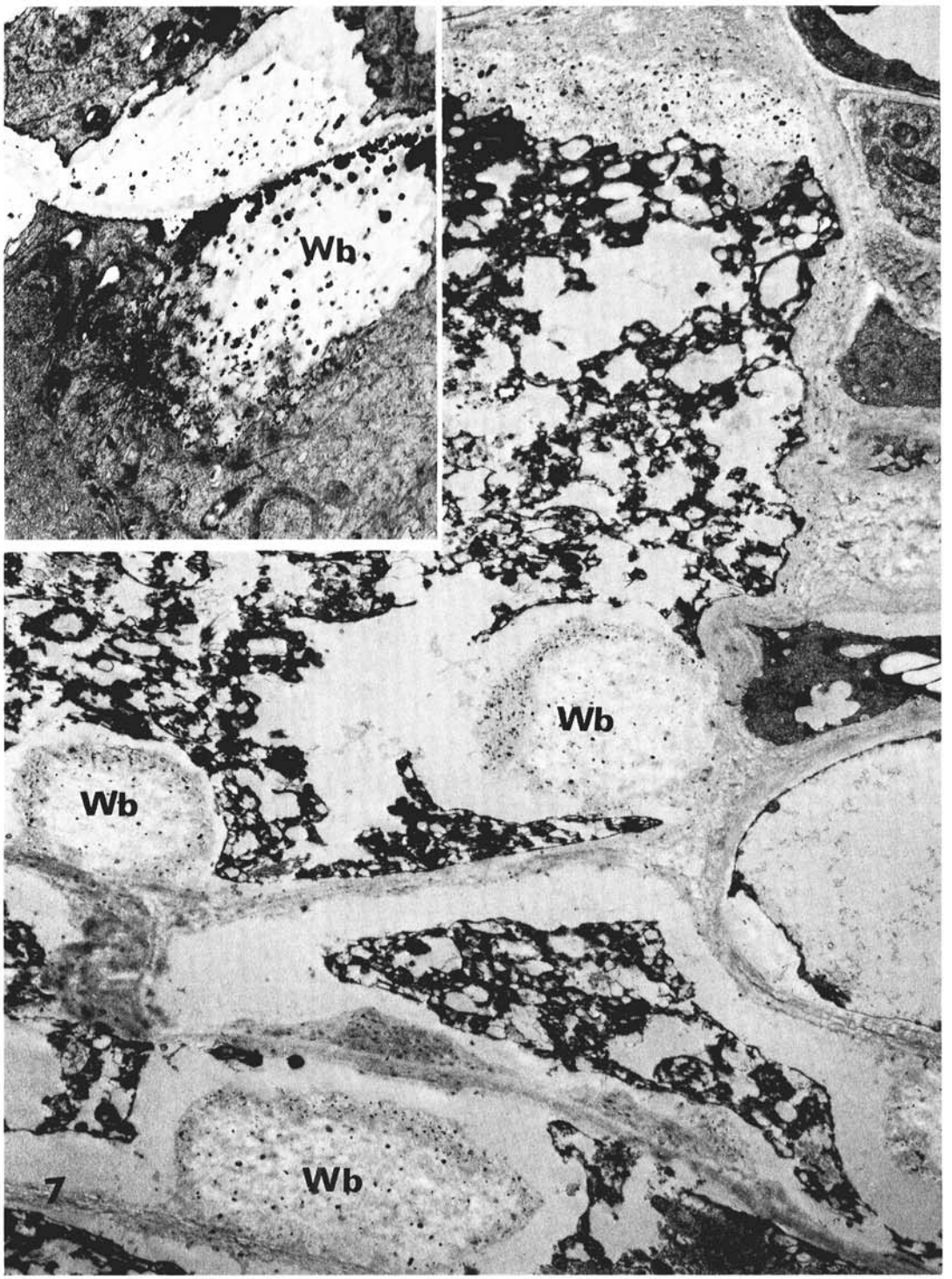


Fig. 7. Irregular cell wall thickenings in a synctium and in adjacent cells 7 days after inoculation. Cytoplasmic degeneration has proceeded until cell organelles are no longer identifiable (× 6,000). (Inset) Thickened wall of syncytium 7 days after inoculation. A large number of microtubules are accumulated at and directly associated with the base of a bulbous wall thickening. Electron-dense particles of various sizes are randomly distributed in the wall thickenings (× 12,000). Mt = microtubule; Wb = cell wall thickenings.

Fig. 8. Nematode adjacent to syncytium 7 days after inoculation. Cell wall is extremely thickened; some structure is still evident in nematode (× 12,000). Ne = nematode S = stylet; Wb = cell wall thickenings.

indicates that the initial stages are very similar to the syncytia formed in susceptible Lee soybean roots (4). Syncytia are apparently formed as a response to the feeding of the nematode larvae. However, syncytia in Peking did not reach the size of syncytia in Lee, and cell wall thickenings were much more prominent in Peking. Microtubules were more numerous in syncytia in Peking than in Lee, and were concentrated near the areas of cell wall thickenings. In our studies, penetration of the syncytium by the nematode stylet was observed (Fig. 6) in Peking but not in Lee.

Deposition of secondary wall material to wall off the diseased area may be the mechanism of resistance to *H. glycines*. Boundary formations or paramural bodies with associated microtubules appeared early in development of the syncytium in resistant Peking, but were not apparent in susceptible Lee (4). Esau et al. (3), Robards (11), and Marchant & Robards (6) reported that boundary formations or paramural bodies (11) were associated, in function, with the deposition of cell wall material. Secondary wall thickenings completely walled off the plasmodesmata. It is, therefore, reasonable to assume that boundary formations or paramural bodies observed in this study are associated with the abnormal cell wall thickening which later completely walled off the plasmodesmata. This would prevent cell to cell movement of materials which might lead to a deficit in food supply or buildup of toxic by-products within syncytia.

The increase in lipidlike globules in degenerating syncytia may indicate an increase in hydrolytic enzyme activity. Matile & Spichiger (7) reported that lipid-rich cytoplasmic bodies contain hydrolytic enzymes. Activity by these enzymes could result in cytoplasmic degeneration of a group of cells in which the by-products could not be eliminated. As a result, an accumulation of toxic materials would kill the nematode.

Huang & Maggenti (5) observed secondary wall thickenings on the inside of giant cells in *Vicia faba* and *Cucumis sativus* infected by *Meloidogyne javanica*. However, the thickenings were irregular and did not block the plasmodesmata.

In Peking soybean, the situation is similar to the local lesion reaction of viruses in certain hosts where an area of dead tissue is also surrounded by a thickened secondary wall. Spencer & Kimmins (13) studied the ultrastructure of local lesion formation by tobacco mosaic virus in Pinto beans, and Tu & Hiruki (15), did a similar study of potato virus-M in Red Kidney beans. In both cases, the cell walls surrounding the infected areas became thickened. These thickenings also appeared to seal off the plasmodesmata, thus preventing the cell to cell movement of virus particles from the infected cells to the surrounding tissues.

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