# An Ultrastructural Study of Syncytium Development In Soybean Roots Infected with Heterodera glycines

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ABSTRACT

Syncytium formation in Lee soybean roots infected with *Heterodera glycines* was studied using the electron microscope at 42 hr, 4, 7, and 15 days after inoculation. Cell wall perforations appeared to be responsible for syncytium formation. Abnormal perforations in cell walls were noticeable within 42 hr after inoculation. The size of the perforation increased with time after inoculation. At 15 days, component cells were no longer distinguishable. Vacuoles decreased in size with increasing age of syncytium, indicating that an increase in cytoplasm replaced the central vacuole. Plastids were abundant in early stages of syncytium formation, and decreased in number at later stages. Endoplasmic reticulumlike material increased with age of the syncytium, and was arranged in a parallel manner in older syncytia. Phytopathology 61:347-353.

Syncytium development in soybean roots infected with the soybean-cyst nematode, *Heterodera glycines*, was described at the light microscope level by Endo (4) who found that syncytia develop within 1 day of penetration by second-stage larvae. Cells penetrated by the stylet of the nematode showed cellular hypertrophy, cell wall dissolution, and clumping of nuclei from contiguous cells.

Syncytium development in roots infected with root knot nematode has been studied with light microscopy and electron microscopy (2, 3, 5, 6, 7). Bird (2) studied the ultrastructure of *Meloidogyne javanica*-induced syncytia, and attributed syncytium formation to a combination of cell wall breakdown and mitosis, accompanied by thickening of surrounding syncytium walls and an increased density and quantity of the cytoplasm. Huang & Maggenti (6, 7), however, maintain that mitosis without subsequent cytokinesis occurs in syncytium development, and that cell wall breakdown plays no part in the development of *Meloidogyne javanica*-induced syncytia.

This study reports the ultrastructure of syncytia in soybean roots infected with the soybean-cyst nematode, *Heterodera glycines* (Ichinohe, 1952), at various stages in their development.

MATERIALS AND METHODS.—Soybeans (*Glycine max* [L.] Merr. 'Lee') were planted in white quartz sand and grown in the greenhouse for 7 days. They were inoculated on the 7th day with second stage larvae of *H. glycines* by the method of Riggs & Hamblen (10). Roots were exposed to penetration for 24 hr, then washed and the plants repotted in sand. Forty-two hr, and 4, 7, and 15 days after inoculation, roots were washed and fixed for 2 hr in 6.25% glutaraldehyde in 0.025 M phosphate buffer at pH 7.2. The roots were then washed for a total of 30 min in three changes of 0.025 M phosphate buffer and postfixed for 2 hr in 1% osmium tetroxide in buffer of the same molarity. After a distilled water wash, small portions of roots containing nematodes (nematodes were obvious after osmium tetroxide fixation) were excised. This tissue was stained overnight in a 0.5% solution of uranyl acetate (1), dehydrated in an ethanol series, and embedded in Epon. Sections cut 1 μ thick with glass knives on an LKB-ultratome were stained with toluidine blue (11) and viewed with a light microscope to verify presence of syncytia. When a syncytium was present, sections were cut at 600-800 Å from the adjacent tissue, stained with 2% uranyl acetate followed by lead citrate (9), and viewed with a Siemens Elmskop 1A electron microscope.

RESULTS.—During early stages of infection, the nematode or cells modified by the nematode were used to locate syncytia. The head region was located intracellularly near the syncytium site in all cases (Fig. 1, 3). Cells containing the nematode and nonsyncytial cells directly adjacent to the nematode showed severe mechanical damage, i.e., cell wall breakage and cytoplasmic and nuclear disintegration (Fig. 1, 3). This type of cell destruction served as a reliable indicator of nematode presence.

Forty-two hr after inoculation, cell wall perforations were evident in the affected cells (Fig. 2, 3). The ends of the wall fragments were rounded, not jagged or sharp. The cells which were affected by feeding of the nematode showed increased cytoplasmic density. The affected cells contained numerous plastids and large amounts of endoplasmic reticulumlike material (Fig. 2, 3). Large central vacuoles were reduced in size. This was strikingly demonstrated when a comparison was made between the central vacuole of cells from the vascular cylinder of noninfected roots (Fig. 7) and those of nematode-affected cells (Fig. 2, 3). In some cases, the nuclei of nematode-affected cells were lobed and contained prominent nucleoli (Fig. 2, 3).

Cell wall perforations were numerous and pronounced 4 days after inoculation (Fig. 4). The distance between cell wall fragments was greater than in the

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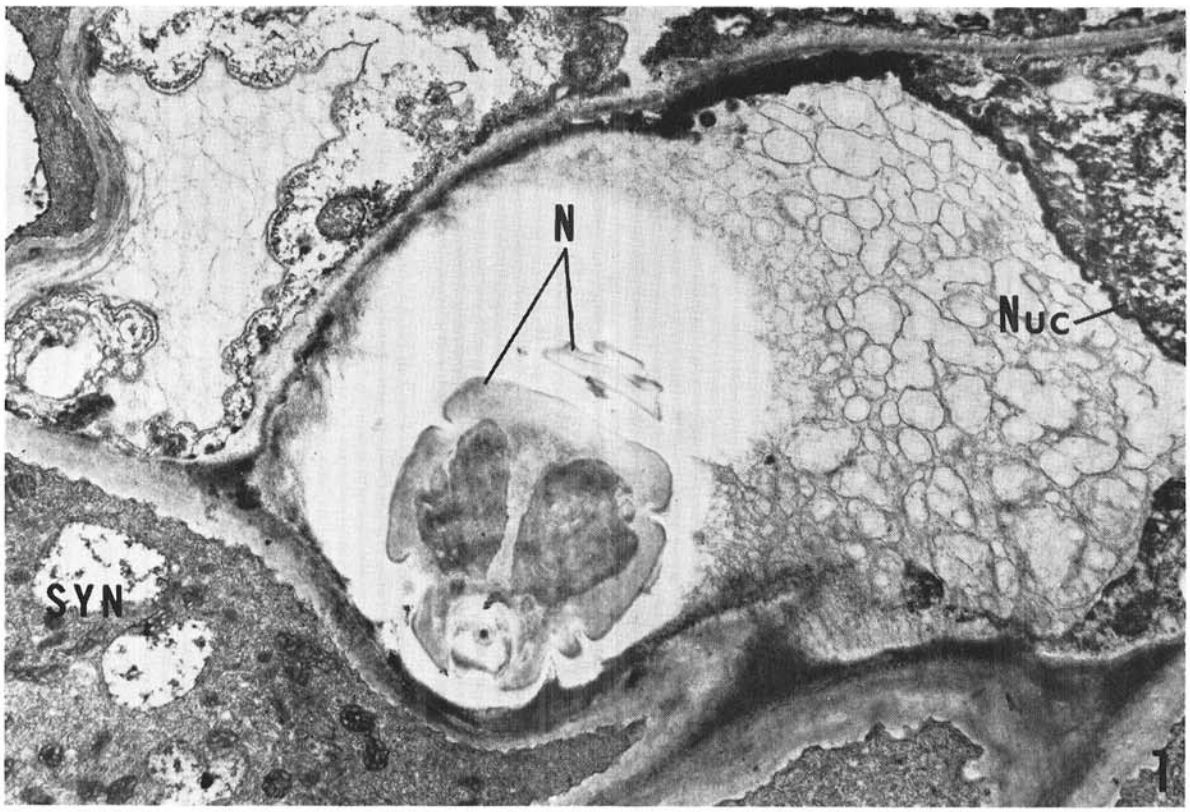
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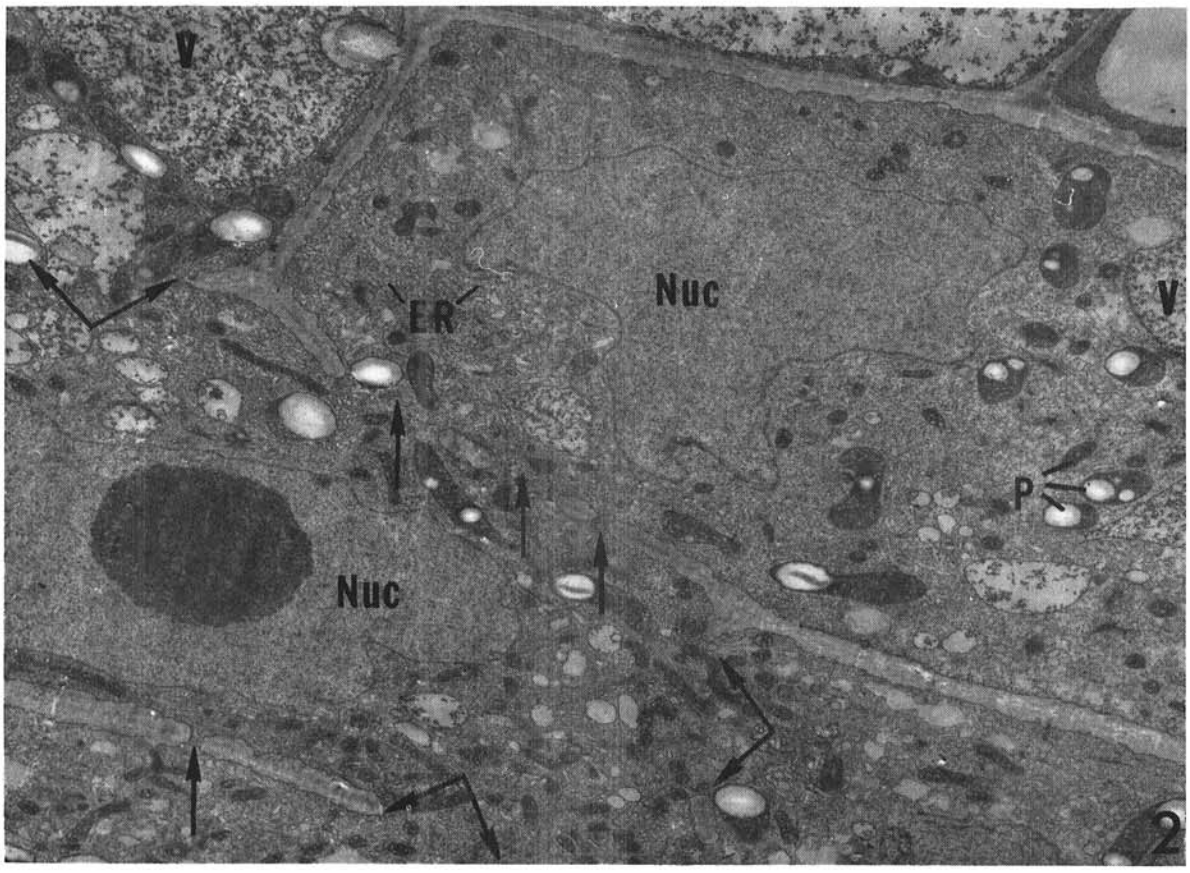
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**Fig. 1-2.** **1)** Anterior portion of *Heterodera glycines* (N), in a soybean root cell located near the feeding site of a syncytium (SYN). Note the severe damage to the cell containing the nematode and adjacent cell. These cells show nuclear (Nuc) and cytoplasmic disintegration. Forty-two hr after inoculation (×6,000). **2)** Portion of a 42-hr syncytium induced by *Heterodera glycines* in soybean root, showing numerous cell wall perforations (→), plastids (P), vacuoles (V), abundant endoplasmic reticulumlike material (ER), and cytoplasm which is continuous from one cell to another through the perforations (×4,000).

←

42 hr syncytia. Cytoplasm of syncytium component cells was continuous through the spaces between cell wall fragments. The number of cells involved in the syncytium had increased. Vacuoles were smaller than those found in 42 hr syncytia and in adjacent normal tissue (Fig. 4).

At 7 days, portions of syncytia were easily recognized with the electron microscope. Cells contributing to the syncytium were no longer distinguishable, although occasionally cell wall fragments were arranged in the cytoplasm to indicate the original cell wall boundary (Fig. 5). The size of the syncytium had greatly increased. A comparison of the size of a portion of the syncytium in Fig. 5 with the adjacent, intact vacuolated cell indicates the magnitude of the syncytium. Syncytia were completely filled with cytoplasm containing a few small vacuoles (Fig. 5). The most prominent change in the cytoplasm at this stage was the increased amt of membranous material which resembled smooth-surfaced endoplasmic reticulum. In some areas, this endoplasmic reticulum showed parallel arrangement (Fig. 5). The number of plastids per unit area were fewer than in the cytoplasm of a 4-day syncytium (Fig. 5).

In contrast to the 7-day infections, there was a definite increase in endoplasmic reticulumlike material in the syncytia cytoplasm after 15 days (Fig. 6, 8). The parallel appearance was more pronounced, the proliferated smooth-surfaced endoplasmic reticulum appeared swollen, and the diam of the cisternae had increased in size.

In all samples, nuclei of syncytia were large and contained prominent nucleoli (Fig. 2, 3, 6). In only one instance was a mitotic figure observed in a syncytium. No clumping of nuclei was evident.

DISCUSSION.—Cell wall breakdown appears to be responsible for syncytium formation. The nature of this cell wall breakdown is not known at the present time. If the breakdown were caused by mechanical force of the nematode or expanding cytoplasm, edges of the wall surfaces would be sharp, and breakage probably would occur in only one location. The ends of the cell walls were rounded, however, and perforations occurred in a number of locations in close proximity. Enzymatic breakdown thus appears more likely, and either continued cell wall degradation or the gradual increase in amt of cytoplasm causes the distance between cell wall fragments to increase. Fusion of the protoplasts evidently occurs, since no plasmalemma is present between the protoplasts in the area of cell wall perforation.

The hypothetical significance of decreased size and number of plastids and increased endoplasmic reticulumlike material is in the relationship between cytoplasmic increase and "food" production for the nematode. This relationship is indicated, as the period of hypertrophy coincides with the period of nematode growth (8). At 15 days the hypertrophy may have ended, as the swollen condition of the endoplasmic reticulumlike material and absence of plastids indicate a state of degradation.

The results of this study agree with Endo's light microscope study (4) that cell wall dissolution and cellular hypertrophy function in syncytium formation; however, no clumping of nuclei of contiguous cells was observed.

A comparison of these results to studies of *Meloidogyne*-induced syncytia (2, 6, 7) indicates some similarities between the two types of syncytia. Root knot-induced syncytia have been described as containing "dense" cytoplasm with an abundance of endoplasmic reticulum and large nuclei with prominent nucleoli (2). These characterizations apply to soybean cyst nematode-induced syncytia as well. The so-called "boundary formations" noted by Huang & Maggenti (6) studying root knot infections were found in a few instances in this study (Fig. 6), but to a limited extent. The conflict in the mechanism of syncytial formation for root knot infections reported by Bird (2) and by Huang & Maggenti (6, 7) should be resolved by further investigations.

Cell wall breakdown appears to be the mechanism for syncytium development in soybeans infected with *H. glycines*, and mitosis without cytokinesis seems not to be a major factor.

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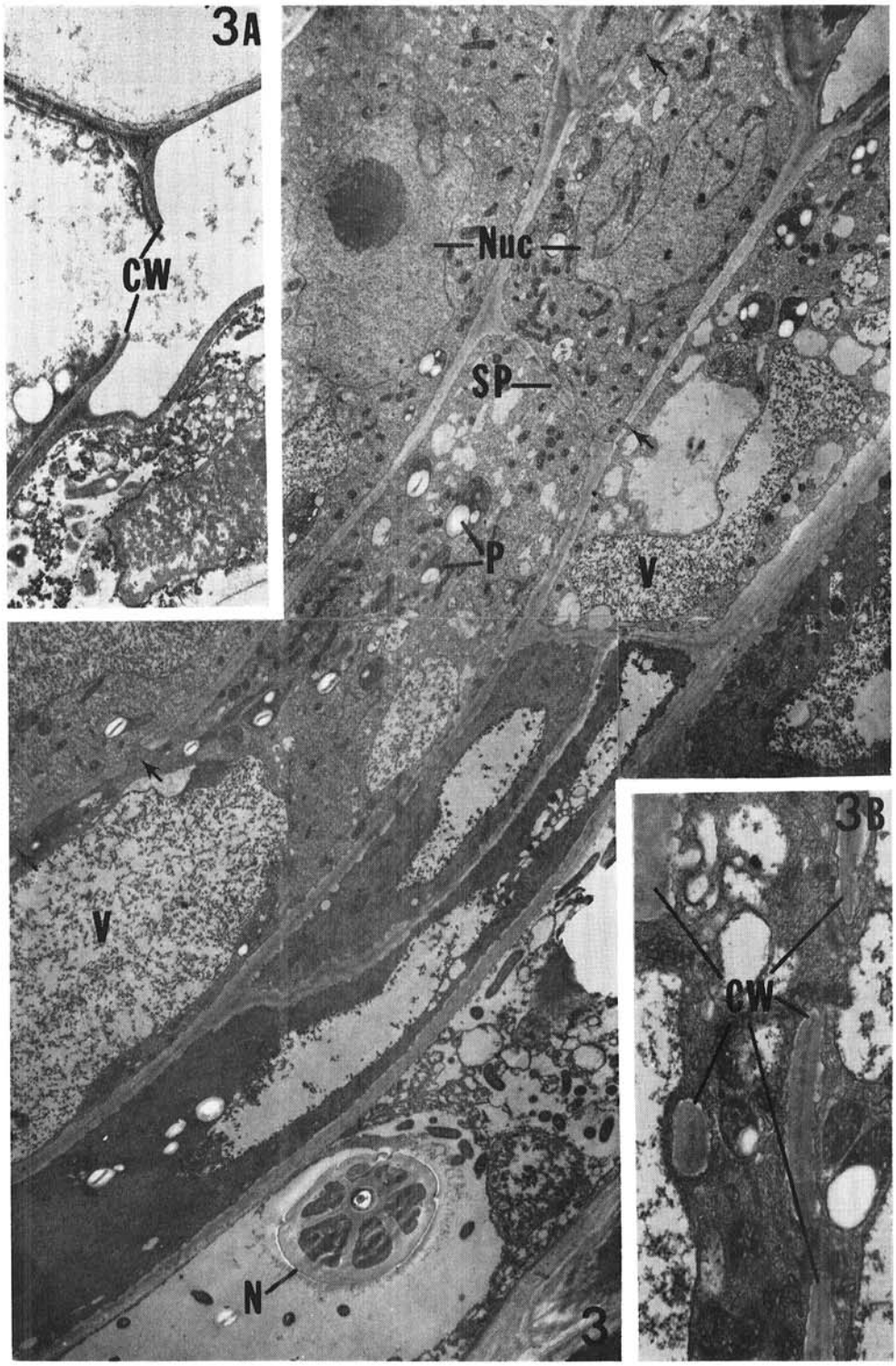
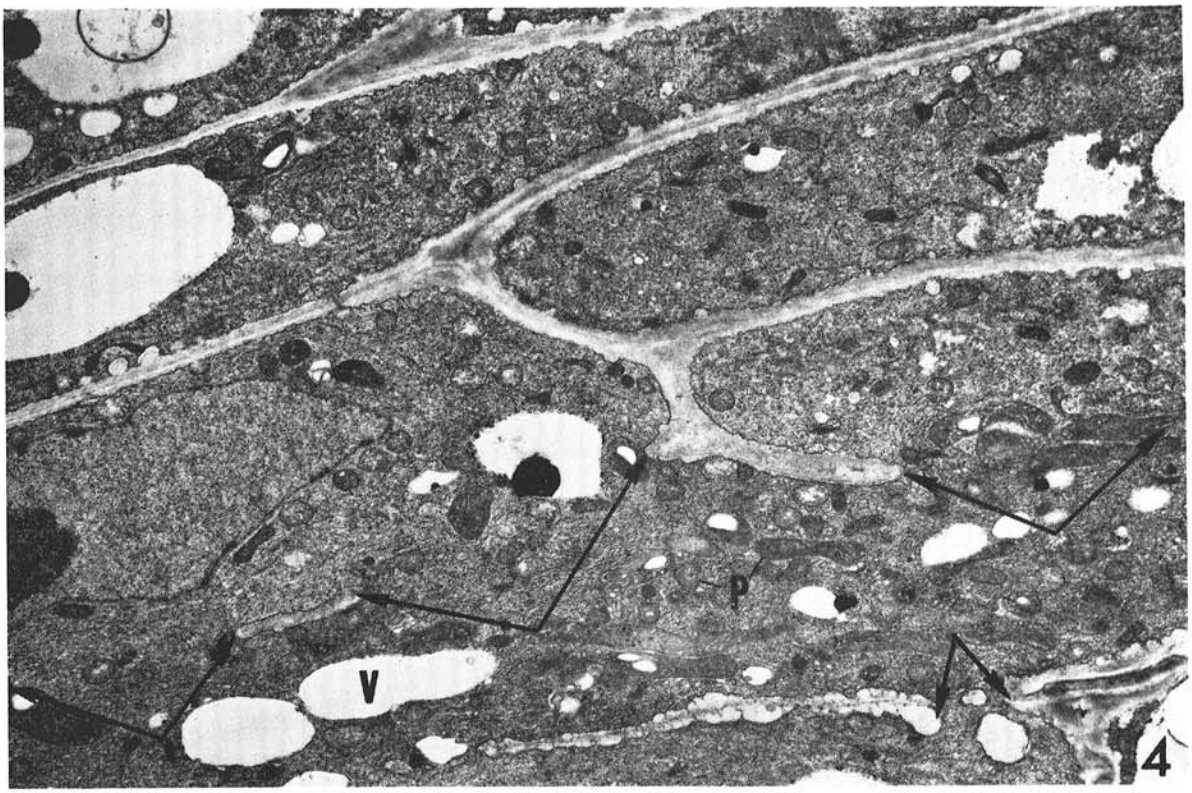



**Fig. 3.** *Heterodera glycines* (N) in relation to early stage of syncytium in soybean root. Arrows indicate cell wall perforations. Note the abundance of plastids (P) in the affected cells. Nuclei (Nuc) of the syncytium are lobed in some cases and contain prominent nucleoli. Present in this syncytium is a sieve plate (SP) and large vacuoles (V). Forty-two hr after inoculation (×3,000). **A)** Cell wall (CW) breakage due to mechanical forces of nematode invasion. Note the sharp, abrupt ends of the broken cell wall and compare these to figure 3B (×4,000). **B)** Higher magnification of cell wall (CW) perforations in syncytium. Note the rounded appearance of the ends of the cell wall (×12,000).



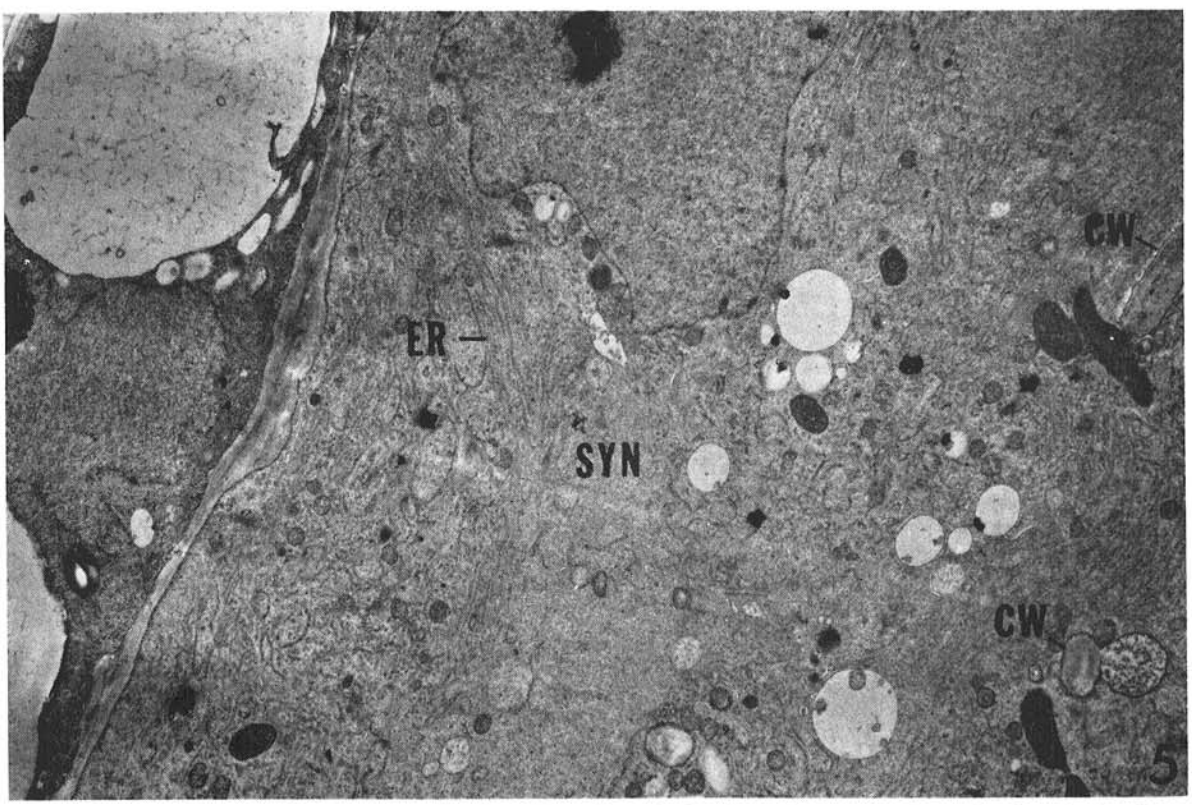
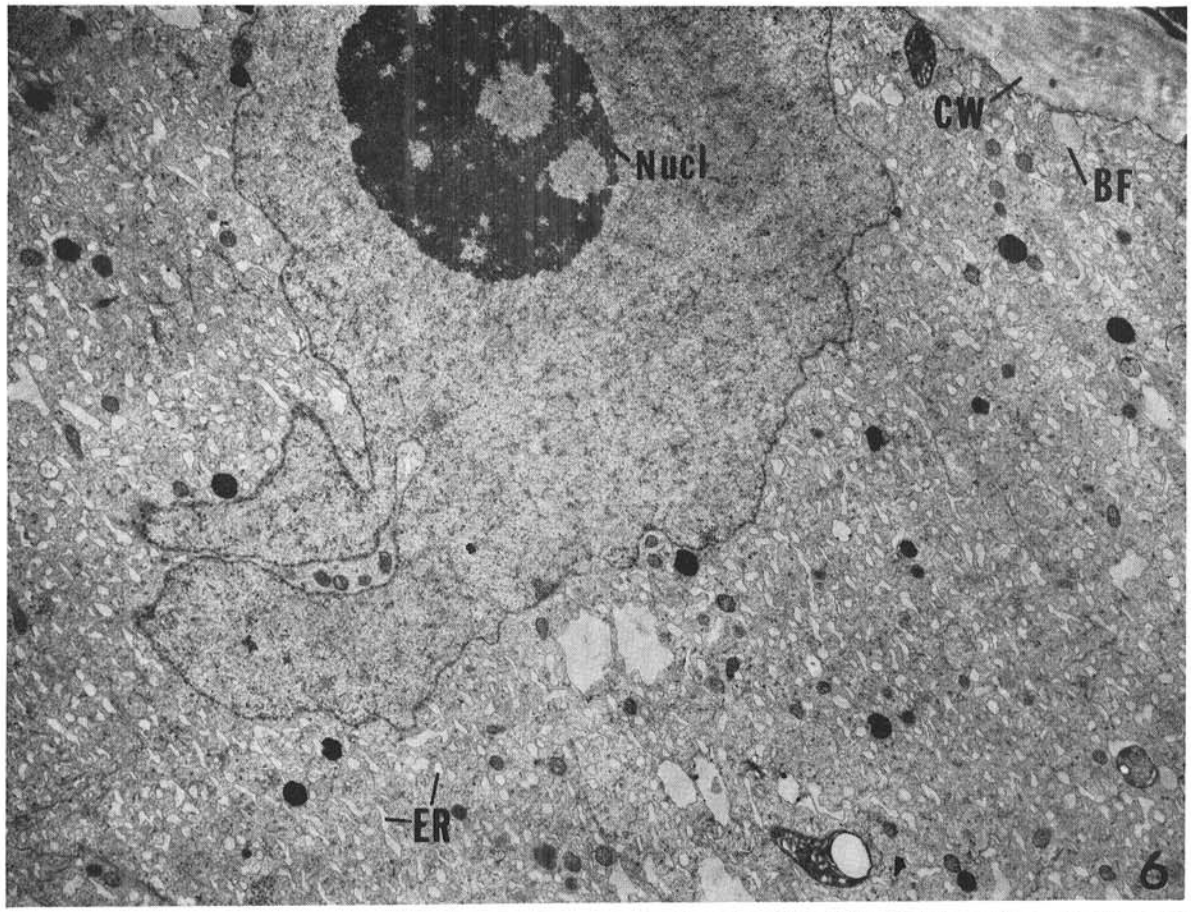
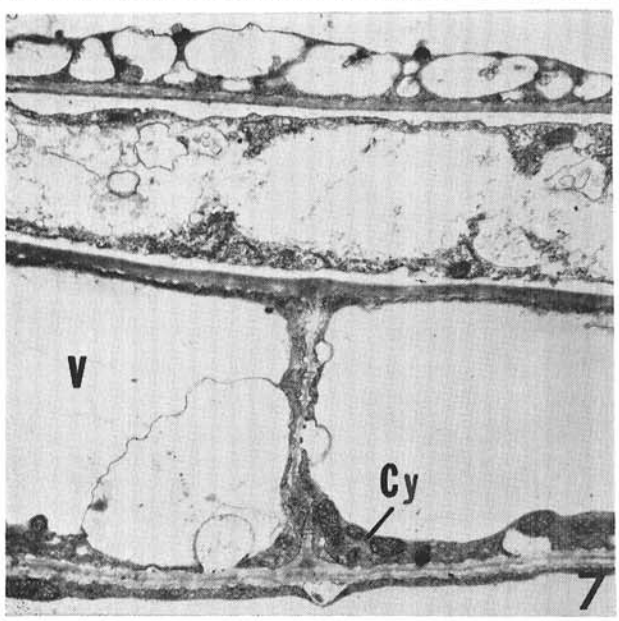




**Fig. 4-5.** **4)** Portion of a syncytium induced by *Heterodera glycines* in soybean root 4 days after inoculation. Large areas of cell wall are absent (→), vacuoles (V) are small, plastids (P) are abundant (×6,000). **5)** Portion of a syncytium (SYN) 7 days after inoculation and an adjacent, intact, vacuolated cell. Note the cell wall (CW) fragments and the parallel arrangement of the endoplasmic reticulumlike material (ER) in the syncytium. Vacuoles and plastids are less numerous than in 4 day syncytia (×4,800).



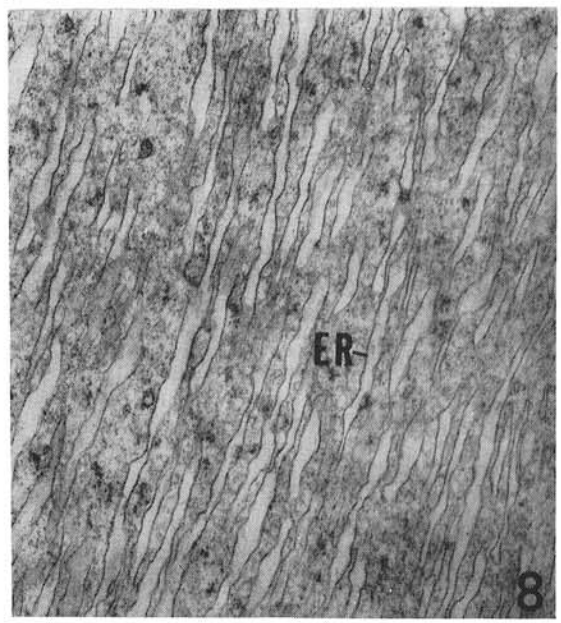




**Fig. 6-8.** **6)** An area of a 15-day syncytium induced by *Heterodera glycines* in soybean root. The quantity and appearance of the smooth-surfaced, endoplasmic reticulumlike material (ER) is striking. Note the thickened cell wall (CW), the large nucleus with its prominent nucleolus (Nucl), and the so-called boundary formation (BF). Vacuoles and plastids are less obvious than in a 7 day syncytium (×4,000). **7)** Control cells of soybean root vascular cylinder with large vacuoles (V) and a thin layer of cytoplasm (Cy) adjacent to cell wall (×6,000). **8)** A higher magnification of the parallel-oriented, smooth-surfaced, endoplasmic reticulumlike material (ER) of 15-day syncytium induced by *Heterodera glycines* in soybean root (×16,000).

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