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Effects of Hydroxyurea on the Ultrastructure of Giant Cells in Galls Induced by *Meloidogyne javanica*[1]

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Abstract: Hydroxyurea (HU) at concentrations of 10 or 20 mg/liter was included in a medium on which excised tomato roots infected with the root-knot nematode *Meloidogyne javanica* were grown. In the HU-treated roots, giant cells were small and contained large vacuoles. Giant cell nuclei were amoeboidal with relatively small nucleoli in treated roots, compared with giant cells of nontreated galls. In treated-root giant cells, the cytoplasm was diffuse and few organelles such as mitochondria, dictyosomes, and endoplasmic reticulum were detected; also, walls of giant cells were thin with less extensive ingrowths than in nontreated roots. We conclude that HU suppressed normal giant cell formation interfering with its function as a feeding cell.

Key words: induced resistance, cytoplasm, nucleus, cell wall ingrowths, vacuoles.

Orion et al. (15) reported that high concentrations of ammonium nitrate applied to *Meloidogyne incognita* cultures on excised tomato roots suppressed giant cell development and thus indirectly hampered nematode development. More recently Glazer and Orion (8) demonstrated that hydroxyurea (HU) at rather low concentrations also suppressed development of *M. javanica* in excised tomato roots in culture. The fact that HU damaged the compatibility between the host and the nematode similar to the hypersensitive reaction occurring naturally in root-knot nematode resistant plants (7,18) suggested that HU induces resistance in susceptible tomato roots. In the greenhouse, HU applied as an aqueous soil drench suppressed giant cell formation and development of *M. javanica* on various hosts in four soil types (9). Furthermore, high soil temperature nullified the effect of HU (8) in much the same way as the genetic hypersensitive reaction in *M. javanica*-resistant tomato cultivars is broken (3), thus supporting the idea that HU induces plant resistance to nematodes.

We report here the ultrastructural changes occurring within giant cells induced in tomato roots by *M. javanica,* as influenced by HU application.

Materials and Methods

Roots of tomato, *Lycopersicon esculentum* cv. Hosen Eilon, were grown in petri dishes on a chemically defined basal medium and were inoculated with egg masses of *M. javanica* as described previously (15). HU was incorporated into the medium at various concentrations prior to autoclaving. Galls and root segments were collected 3–4 weeks after nematode inoculation from petri dishes flooded with 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 25 C to fix the tissues. Roots were transferred to vials containing fresh fixative and after 2 hours were washed in six changes of buffer over another 2 hours. Roots were then postfixed in 2% buffered osmium tetroxide for 2 hours, dehydrated in an acetone series, and embedded in Epoxy resin, Agar 100 resin (Agar Aids, Essex, England). Ultrathin sections were cut with glass knives on an LKB IV Ultramicrotome, stained with uranylacetate followed by lead citrate, and examined with a JEOL electron microscope JEM-100 CX II at 80 kV. Thick sections (1 μm) of material embedded for EM were cut as mentioned above and stained with toluidine blue.

Results

Light microscopy of 4-week-old nontreated gall sections revealed typical giant cells around the nematode head, characterized by dense cytoplasm, many enlarged nuclei with prominent nucleoli, and thick cell walls (Fig. 1A). Deformed xylem was adjacent to the giant cells. In comparison, giant cells in roots exposed to HU at both 10 and 20 mg/liter were smaller and contained many vacuoles of various sizes (Fig.

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1B, C). At 10 mg/liter HU giant cells were usually partially filled with vacuoles of varying sizes, whereas at 20 mg/liter they were completely filled with large vacuoles. In many cases, giant cells virtually failed to develop in the presence of HU at 20 mg/liter (Fig. 1D); deformed xylem elements adjacent to giant cells were similar to those in nontreated nematode infected roots.

Electron microscopy revealed that the nuclei in nontreated galls were enlarged and somewhat lobed and possessed nucleoli (Fig. 2A). In the presence of HU, nuclei in giant cells were amoeboid and possessed relatively small nucleoli (Fig. 2B) and giant cell cytoplasm was diffuse and contained many large vacuoles (Fig. 2C, D). In the absence of HU, giant cell cytoplasm contained a dense population of cell organelles, such as mitochondria, endoplasmic reticulum (ER), and dictyosomes (Fig. 3A). Giant cells developed in the presence of HU contained a low density of mitochondria and ER and many vacuoles filled with electron dense material; tonoplasts were more intensely stained than in the absence of HU in the medium (Fig. 3B).

The structure of giant cell walls was also strongly affected by HU. In the absence of HU, giant cell walls were thick with many deeply penetrating cell wall ingrowths (Fig. 4A), whereas in the presence of HU at 10 mg/liter, giant cell walls were thin and cell wall ingrowths poorly developed (Fig. 4B). At 20 mg/liter HU, there was no thickening of giant cell walls and few tubular cell wall ingrowths were seen (Fig. 4C).

Discussion

Our findings confirm again that giant cells induced by root-knot nematodes on susceptible excised roots growing in vitro and giant cells in the intact roots of plants growing in soil are similar. Giant cells are large and multinucleate, contain dense cytoplasm rich with cell organelles, and possess extremely thick cell walls with deeply invaginated ingrowths. These features are evidence of high metabolic activity induced by the nematode to supply its enormous demands for nutrients (4). Jones (10–12) noted that giant cells function as transfer cells characterized by the cell wall ingrowths that allow rapid uptake of solutes destined for the nematode. Normal nematode development depends on successful formation of its feeding site, the giant cell (5).

In the presence of HU, nematodes failed to induce normal giant cells, giant cells were small, and their cytoplasm contained few membraneous organelles and was highly vacuolated. Giant cell nuclei were abnormally amoeboid, and their cell walls were thin with sparsely developed cell wall ingrowths.

We conclude that the ultrastructural changes in giant cells exposed to HU are cytological evidence of marked interruption of normal giant cell metabolic activities. Thus, the demands of the nematodes for nutrients are not satisfied. Indeed, many nematodes in HU-treated plants fail to mature (8,9). Our observations support previous findings on the role of HU in in-

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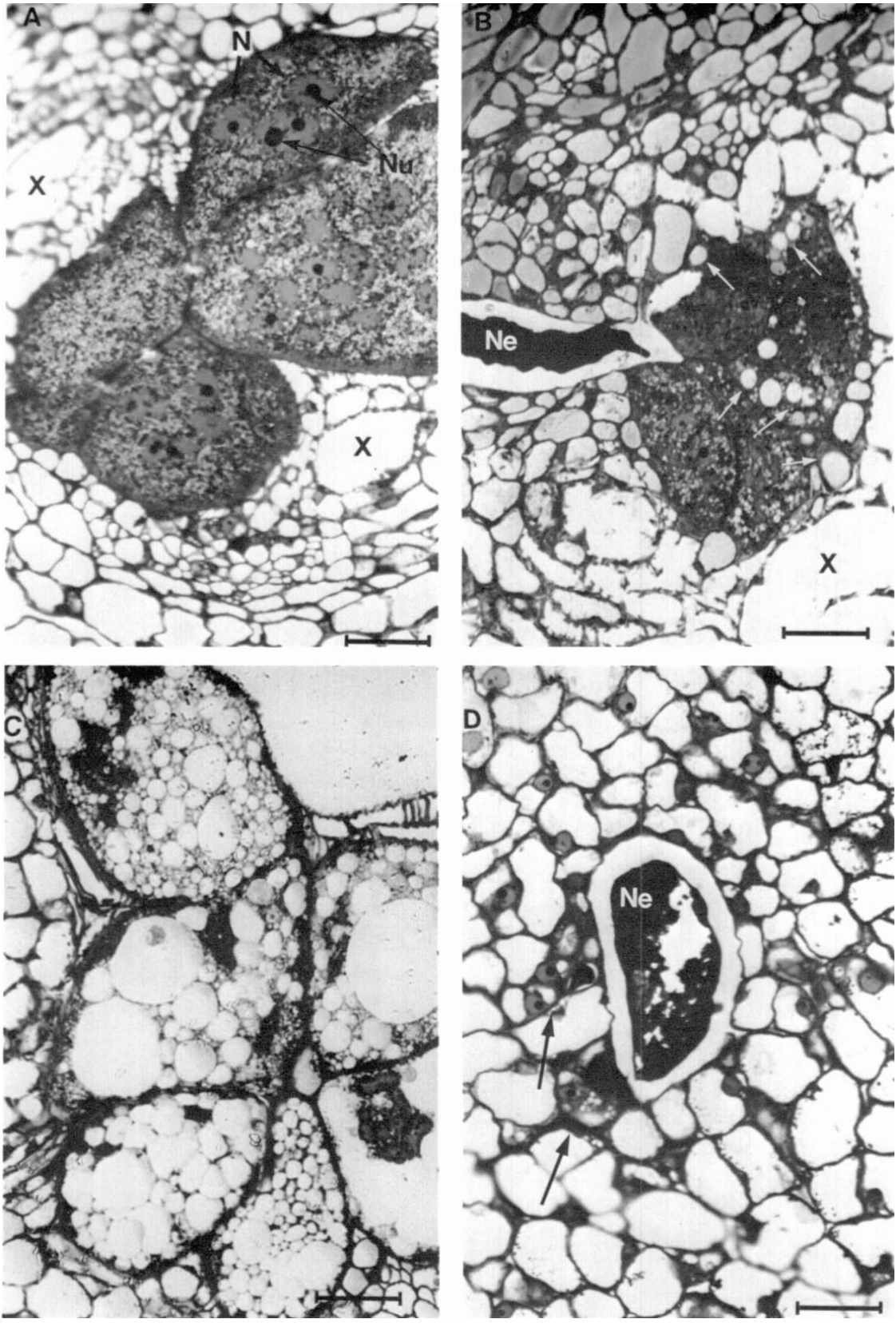
FIG. 1. Light micrographs of cross sections of *Meloidogyne javanica*-induced galls in tomato roots. A) Nontreated control. Typical giant cell with dense cytoplasm, irregularly thickened cell walls, and swollen nuclei (N) with enlarged nucleoli (Nu). B) Treated with hydroxyurea at 10 mg/liter. Note the small giant cell, nuclei and nucleoli, the thin cell walls, the vacuoles (arrows) of various sizes, and the nematode (Ne). Deformed xylem vessels (X) adjacent to the coenocyte are seen in A and B. C) Giant cells completely filled with large vacuoles. No wall thickenings are evident. D) Arrows point to the few cells which seem to be affected by the nematode (Ne). C and D treated with hydroxyurea at 20 mg/liter. Bars = 20 μm.

FIG. 2. Electron micrographs of *Meloidogyne javanica*-induced giant cells in tomato roots. A) Nontreated control. Enlarged nucleus (N) with prominent nucleolus (Nu). B) Amoeboid nucleus (N) of giant cell in gall treated with hydroxyurea at 10 mg/liter. C) Treated with hydroxyurea at 20 mg/liter. D) Amoeboid nucleus of giant cell in a gall treated with HU at 20 mg/liter. The giant cell is filled with large vacuoles. Note the differences in shape of the nucleus and nucleolus and the size of the vacuoles in the HU-treated galls vs. nontreated galls. Bars = 5 μm.

FIG. 3. Electron micrographs of the cytoplasm of *Meloidogyne javanica*-induced giant cells in tomato roots. A) Nontreated with the usual membranous system as endoplasmic reticulum (ER), dictyosomes (D), and mitochondrion (M). B) Treated with hydroxyurea at 10 mg/liter. Note the virtual absence of membranous systems and the presence of electron dense material within the vacuoles. Bars = 1 μm.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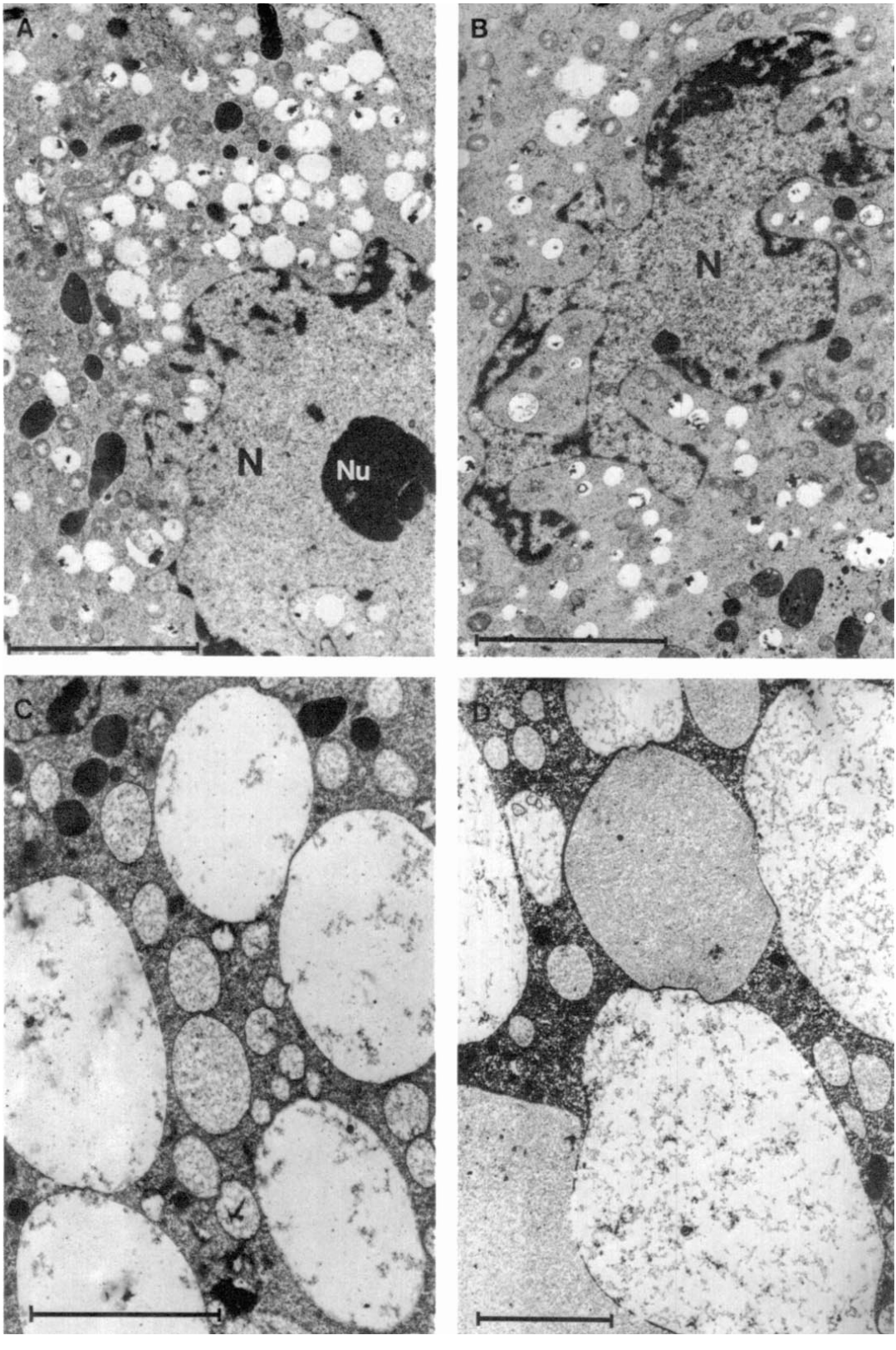
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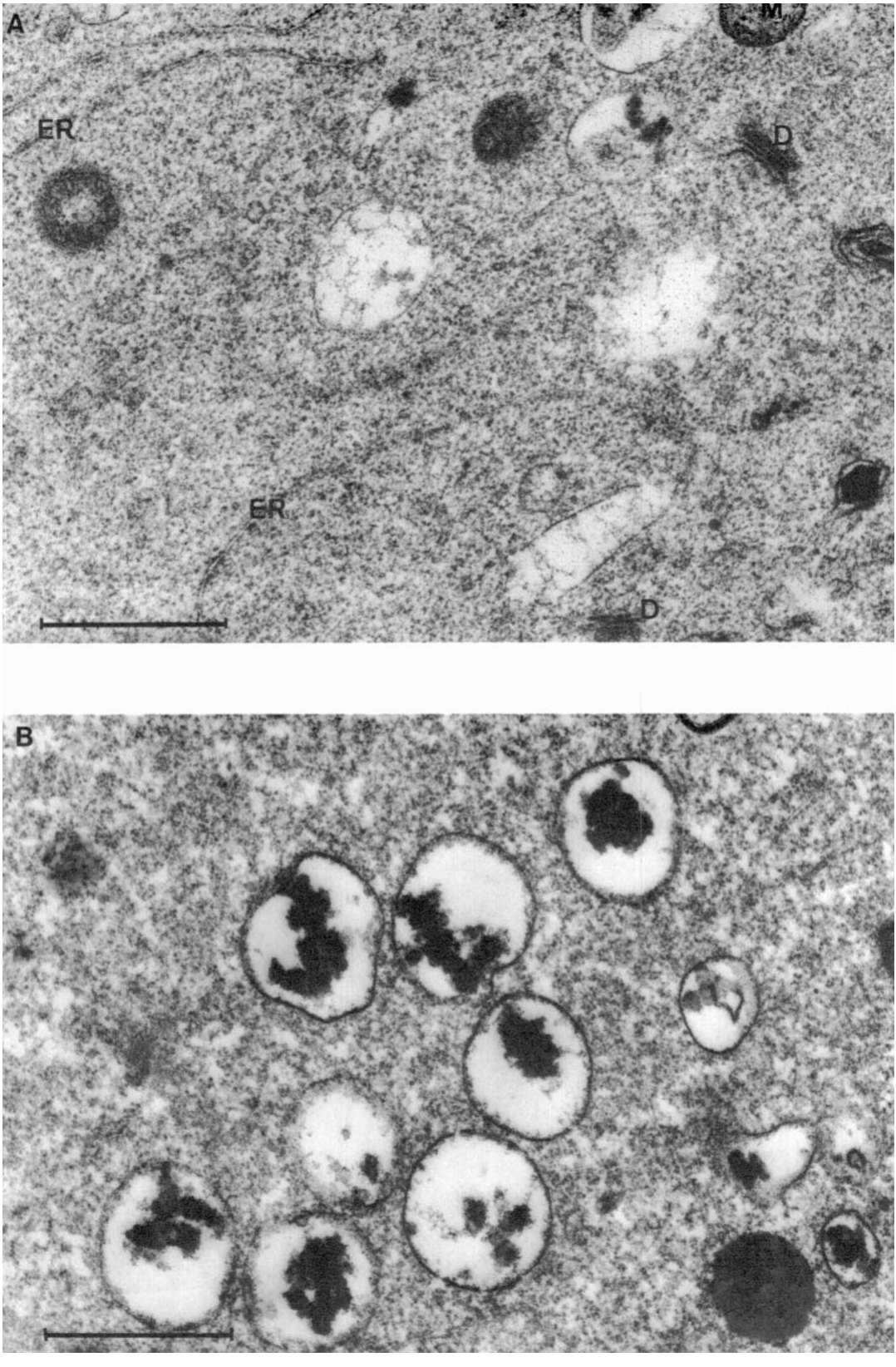
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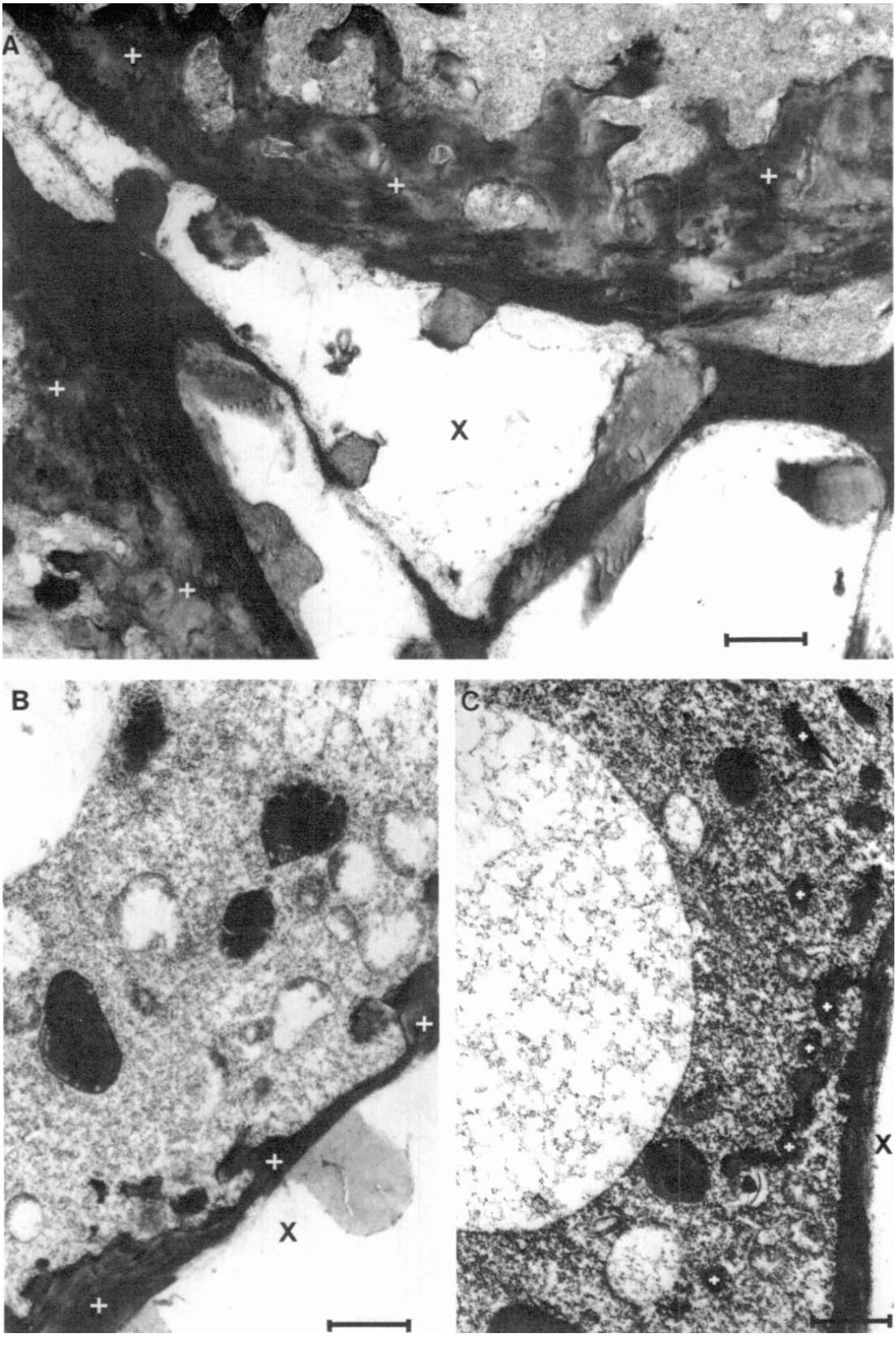
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ducing resistance in plants to root-knot nematodes.

Similarities between induced resistance and the natural hypersensitive reaction have been noted in which giant cells containing large vacuoles with electron dense contents were described early (24–48 hours) in the hypersensitive reaction of resistant tomatoes to *M. incognita* (1,16,19).

Hydroxyurea is an antimitotic and cytotoxic drug acting as a specific inhibitor of DNA synthesis (2,13,14). This activity appears to be caused by inhibition of ribonucleoside diphosphate reductase (2,14), resulting in depletion of the intracellular pool of DNA precursors (14). The biochemical and cytological intensity of HU influence is related to the concentration used, the duration of the exposure, and the sensitivity of the cell system (6,13,14,17). In our study, high concentrations of HU suppressed giant cell development more strongly than did low concentrations. Giant cells were previously shown (8,9) to be more sensitive to HU than apical meristems where DNA is intensively synthesized.

The data presented here confirm and strengthen previous reports (8,9) that HU induces resistance to root-knot nematodes suppressing giant cell development.

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FIG. 4. Electron micrographs of *Meloidogyne javanica*-induced giant cell walls in tomato roots. A) Nontreated with thick cell walls and well-developed wall ingrowths (+). B) Treated with hydroxyurea at 10 mg/liter. Cell walls are thin and have few wall ingrowths (+). C) Treated with hydroxyurea at 20 mg/liter. No thickening of cell walls and the few wall ingrowths (+) are tubular. Xylem vessels (X). Bars = 1 μm.