Ultrastructural modifications of *Meloidogyne javanica* induced giant cells caused by fungal culture filtrates

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SUMMARY

A light and electron microscope study was done of the effect of cell-free culture filtrates of race 1 *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium solani* f.sp. *phaseoli* and *Colletotrichum coccodes* on giant cells induced by *Meloidogyne javanica* in fungal resistant and susceptible tomato *(Lycopersicon esculentum)* cultivars. The ultrastructural modifications of giant cells in *Fusarium* wilt-resistant and susceptible tomato cultivars treated with the filtrates were indistinguishable from those in giant cells of *Fusarium* wilt-infected tomato cultivars. The changes were characterized by membrane and ribosome modifications. Ultrastructural modifications of the giant cells appear to be a direct effect of the fungal culture filtrate and not of the nematode. Giant cells were more sensitive to culture filtrates than were the surrounding parenchyma cells. No toxic effects were observed of fungal culture filtrates on the nematode. The results support the concept that translocatable toxic metabolites from plant pathogenic fungi caused deterioration of *M. javanica* induced giant cells.

RÉSUMÉ

Modifications ultrastructurales causées par les filtrats de cultures de champignons aux cellules géantes induites par Meloidogyne javanica

Une étude en microscopie optique et électronique a porté sur l'influence de filtrats de cultures de cellules libres de la race 1 de *Fusarium oxysporum* f. sp. *lycopersici*, *Fusarium solani* f. sp. *phaseoli* et *Colletotrichum coccodes* sur les cellules géantes induites par *Meloidogyne javanica* sur les cultivars de tomate *(Lycopersicon esculentum)* résistants et sensibles à ces champignons. Les modifications ultrastructurales des cellules géantes des cultivars de tomate résistants ou sensibles au wilt fusarien ne sont pas différentes de celles qui se présentent dans les cellules géantes des cultivars de tomate infestés par l'agent du wilt. Les changements observés sont caractérisés par des modifications de la membrane et du ribosome. Ces modifications paraissent être un effet direct du filtrat de la culture fongique, et non du nématode. Les cellules géantes sont plus sensibles aux filtrats de culture que les cellules parenchymatiques qui les entourent. Aucun effet toxique du filtrat de culture fongique envers le nématode n'a été observé. Ces résultats confortent l'hypothèse suivant laquelle les métabolites toxiques provenant de champignons phytopathogènes causent une détérioration des cellules géantes induites par *M. javanica.*

The deterioration of *Meloidogyne javanica* induced giant cells that occurs in *Fusarium oxysporum* f. sp. *lycopersici* infected tomato cultivars at sites distant from invading fungal hyphae is probably caused by translocatable toxic fungal metabolite(s) (Fattah & Webster, 1983). Giant cells are a nutrient source for the nematodes and essential for normal development and reproduction (Bird, 1974). Several nematode-fungus interaction studies have shown that the fungal component of these disease complexes modifies nematode activity (Powell, 1971; Webster, 1985) and, in *Meloidogyne* interactions, lead to decreased nematode reproduction. This is probably due to destruction of giant cells by the invading fungus (Davis & Jenkins, 1963; Ryder & Crittenden, 1965; Littrell & Johnson, 1969). Consequently, the number of nematodes in the soil is decreased.

This study was done to determine if the ultrastructural modifications of nematode induced giant cells of *F. oxysporum* f. sp. *lycopersici* infected plants were the result of toxic metabolite(s) from the fungus, and if metabolites of other fungi could induce similar giant cell deterioration.

Materials and methods

PLANT MATERIAL

Seeds from two isolines of tomato, *Lycopersicon esculentum* Mill, cv. Pearson A-1 IMP which is susceptible

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and cv. Pearson IMP which is resistant to race 1 of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. (source : Peto Seed Co. Inc., Saticoy, California), were surface sterilized and then grown in pots under greenhouse conditions as described previously (see Fattah & Webster, 1983).

Lima beans, *Phaseolus lunatus* L. cv. L-136 (source : Dr. S. D. Van Gundy, University of California, Riverside) were grown in pasteurized peat-loam-perlite mixture (equal parts) and used as hosts for the nematode stock culture.

Nematode and fungal material

Meloidogyne javanica (Treub) Chitwood (source : Dr. S. D. Van Gundy) were increased from a single egg mass and maintained on potted lima bean plants until required. Freshly hatched juveniles were surface sterilized with 0.1 % bis (7-chlorophenyl diguanido) hexane diacetane (Hibitane) for 10 min (Peacock, 1959) before use in experiments.

F. oxysporum f. sp. *lycopersici* race 1 *(Fol)* isolate (source : Dr. E. E. Butler, University of California, Davis), *F. solani* (Mart.) App & Wr. f. sp. *phaseoli* (Burkh.) Snyd. & Hans. *(Fsp)* isolate (source : Dr. H. S. Pepin, Agriculture Canada, Research Station, Vancouver) and *Colletotrichum coccodes* (Wallr.) Hughes *(Cc)* (isolated from tomato roots in the university greenhouse and its identity confirmed by the Biosystematic Research Institute, Agriculture Canada, Ottawa) were maintained as separate cultures on test tube slopes of potato dextrose agar (PDA) stored at 5°.

Preparation of fungal culture filtrate

Fungus from each of the three stock cultures was cultured on PDA in Petri dishes at 22° for one week. Plugs (0.5 cm^2) of thick, hyphal growth from each of the PDA fungal cultures were transferred to 250 ml Erlenmeyer flasks (one per flask) containing 150 ml of autoclaved Tochinai broth. The inoculated broth was incubated at room temperature on a rotary shaker for 30 d after which all the fungal hyphae and spores were removed from the culture by filtering through cheesecloth, centrifuging, and filtering through 0.45 µm Millipore filters. The fungus-free filtrate was stored in sterile, plastic tissue culture flasks at 5° for subsequent use. Undiluted culture filtrate was designated as 100 % and dilutions were made with mineral nutrient solution (Moore, 1974). The pH value and osmolarity of the culture filtrates and test solutions were measured.

Light and electron microscopy

Pieces of root were processed for light and electron microscopy as described previously (Fattah & Webster, 1983). Sections for light microscopy were cut at 2 µm thickness and stained with 1 % toluidine blue O.

The figures are representative of the findings from sections of at least three resin blocks of processed material of each treatment replicate, and each treatment was replicated three times. Each experiment was repeated at least once.

Experiments

(a) Twenty day-old seedlings of *Fol* susceptible and resistant tomato cultivars were inoculated with about 2 000 freshly hatched, surface sterilized juvenile *M. javanica*, and three weeks later root pieces (3-5 cm long) were excised, surface sterilized for 2 min in 1 % sodium hypochlorite solution, thoroughly rinsed in sterile, distilled water and incubated in the dark in 10 ml of either 50 % *Fol* culture filtrate, 50 % Tochinai broth, 50 % nutrient solution (Moore, 1974) or sterile water in small, sterile Petri dishes at 20°. Samples of treated, nematode infected roots were fixed for light and electron microscopy at 3, 6, 12 and 24 h after start of incubation of the excised roots.

(b) Samples of *M. javanica* infected, *Fol* resistant and susceptible tomato roots were excised 2 wk after nematode inoculation and incubated as described above in 100 % solution of *Fol* culture filtrate for 0.5, 1, 3, 9 and 24 h. After incubation, samples (except those incubated for 24 h) were divided into two subsamples. One subsample was transferred to sterile tap water and the other to autoclaved Tochinai broth, then all subsamples were incubated for a total of 24 h and fixed for light and electron microscope examination.

(c) Three weeks after *M. javanica* inoculation of 20 day old *Fol* resistant and susceptible tomato, the root systems of intact plants were carefully washed, surface sterilized for 2 min with 1 % sodium hypochlorite, incubated in glass jars containing either 100 % or 50 % *Fol* culture filtrate, nutrient solution or either 100 % or 50 % Tochinai broth used as the control (aerated by bubbling through filtered air) for 24 h in a growth chamber (16 h photoperiod at 20 ± 1°). Samples of nematode infected roots were processed for microscopic examination as described above.

(d) Nematode infected root pieces of *Fol* resistant tomato were prepared as described above and incubated for 24 h in 100 % of 50 % concentrations of *Fsp*, *Cc*, or *Fol* culture filtrates and, as controls, in Tochinai broth, nutrient solution of 0.025 M phosphate buffer (pH 8.3 and 6.8). The root pieces were then processed for microscopic examination.

(e) Samples of galled root pieces from a 5-week old *M. javanica* infection of *Fol* resistant tomatoes were incubated for 24 h in 100 % culture filtrate of each of *Fol* (pH 6.8 and 7.8), *Fsp* (pH 6.8 and 8.3), and heat treated *Fol* (in water bath at 100° for 30 min) (pH 6.8), or in 0.025 M phosphate buffer (pH 6.8) or sterile tap

water (controls). The culture filtrate pH was adjusted with 1N HCl.

(f) Freshly hatched, surface sterilized *M. javanica* juveniles were incubated in 100, 50 and 25 % *Fol* culture filtrate for 24 h at 20 ± 1°, washed with tap water and inoculated into 20 day-old tomato seedlings. Samples of galled root tissue were fixed for light and electron microscopic examination 30 d after nematode inoculation.

Results

No light microscope changes were observed in *M. javanica* induced giant cells of either *Fol* susceptible or resistant tomato cultivars treated with 50 % of 100 % *Fol* culture filtrate for less than 12 h or 9 h respectively. However, at the ultrastructural level evidence of initial disorganization of giant cell organelles, including nuclear chromatin condensation, loss of membrane distinctness and accumulation of granules in the plastids, was observed after 3 h in 100 % *Fol* culture filtrate (Fig. 1).

By 12 h in 50 % *Fol* culture filtrate, giant cells in the roots of both cultivars were significantly different from those of the controls. Membrane breakdown and electron light areas in the cytoplasm and nucleoplasm were visible (Fig. 2). Some mitochondria appeared electron light and the cristae were breaking down. The plasma membrane was less distinct, and was separated from the giant cell wall leaving an electron-transparent zone between the cell wall and cytoplasm (Fig. 2 A). The tonoplast had lost its integrity (Fig. 2), and most plastids in the giant cells of both cultivars showed breakdown of the outer membrane and the presence within the plastids of crystalline formations similar to phytoferritin (Fig. 2 B).

After 24 h incubation in 50 % *Fol* culture filtrate giant cell deterioration had progressed significantly in both *Fol* resistant and susceptible tomato cultivars. Giant cell nuclei in both cultivars were smaller and more spherical (Fig. 3 A, B) than " normal " giant cell nuclei (Fig. 1 A). The nuclear membrane was swollen and associated with ribosomes (Fig. 3 C, D), chromatin was condensed along the nuclear membrane and large numbers of electron dense inclusions occurred within the nucleus and within the condensed nuclear chromatin (Fig. 3 C, D). The rough endoplasmic reticulum was swollen (Fig. 3 D) vesiculated and occasionally occurred as parallel stacks (Fig. 4 A). The giant cell wall showed no signs of dissolution even after 24 h of filtrate treatment. Giant cells incubated in Tochinai broth or in nutrient solution appeared ultrastructurally normal with the characteristic vacuolated dense cytoplasm and irregularly shaped nuclei (Fig. 4 B, C).

Parallel changes occurred in giant cells of roots treated in 100 % *Fol* culture filtrate except that they occurred earlier. In several instances giant cells in adjacent clusters showed markedly different structure (Fig. 5 A). By 9 h cytoplasmic vacuoles had lost their regular outline, and phytoferritin crystals were visible within plastids. The giant cell cytoplasm and nucleoplasm showed a high degree of electron density (Fig. 6 A, B). Nuclear chromatin was condensed mostly along the nuclear membrane and contained numerous electron dense inclusions with surrounding electron light areas; these latter inclusions occurred scattered throughout the nucleus (Fig. 6 B, C). Nucleoli were less electron dense and less regular in outline (Fig. 6 B, C) than were those of " normal " giant cells in the untreated controls. The nuclei had lost their characteristic irregular shape and were rounded; nuclear membranes were swollen, partially fragmented and covered with ribosomes (Figs 5 B, 6 B, 6 C). Dark inclusions were visible within the nuclear chromatin, some chromatin appeared to have leaked into the cytoplasm through the ruptured nuclear membrane (Fig. 5 B) and the nucleoli were less electron dense and had highly irregular outlines (Fig. 5 B). The number of ribosomes per unit area of the cytoplasm was greater than that found in " normal " giant cells, occurring as either free units or associated with the endoplasmic reticulum. The latter was vesiculated and occurred in regular parallel stacks (Fig. 6 A, B, C). The rough endoplasmic reticulum had more ribosomes per unit surface area (Fig. 6 A, B, C) than that in the giant cells of control roots. The plasma membrane had degenerated and an electron light zone was visible between the cell wall and the cytoplasm (Fig. 6 A). Surrounding parenchyma cells were less obviously affected by *Fol* culture filtrate as manifested by membrane distinctness and integrity of the cellular organelles (Fig. 7 A, B).

Giant cells in both *Fol* resistant and susceptible cultivars incubated in 100 % *Fol* culture filtrate for 24 h were severely broken down, the cytoplasm was electron light and contained remnants of cell organelles and lipid droplets, as compared with that in unaffected giant cells (Fig. 7 C). The giant cell walls appeared unaffected after 24 h incubation in 100 % *Fol* culture filtrate (Fig. 7 D).

When intact root systems were treated with *Fol* 50 % or 100 % culture filtrate no significant differences were observed between the ultrastructural response of giant cells in *Fol* resistant or susceptible tomato. Giant cells showed structural changes under the light and electron microscope similar to those described above for the filtrate treatment of excised roots. Giant cells in roots incubated in nutrient solution or Tochinai broth (controls) revealed characteristic ultrastructural features typical of *M. javanica* induced giant cells.

Incubation of resistant tomato cultivar root pieces in culture filtrates of *Fol, Fsp,* and *Cc* caused ultrastructural modifications of giant cells similar to those described above for *Fol* culture filtrates. The destruction of giant cells was more severe when root samples were incubated in 100 % than in 50 % culture filtrate. Under

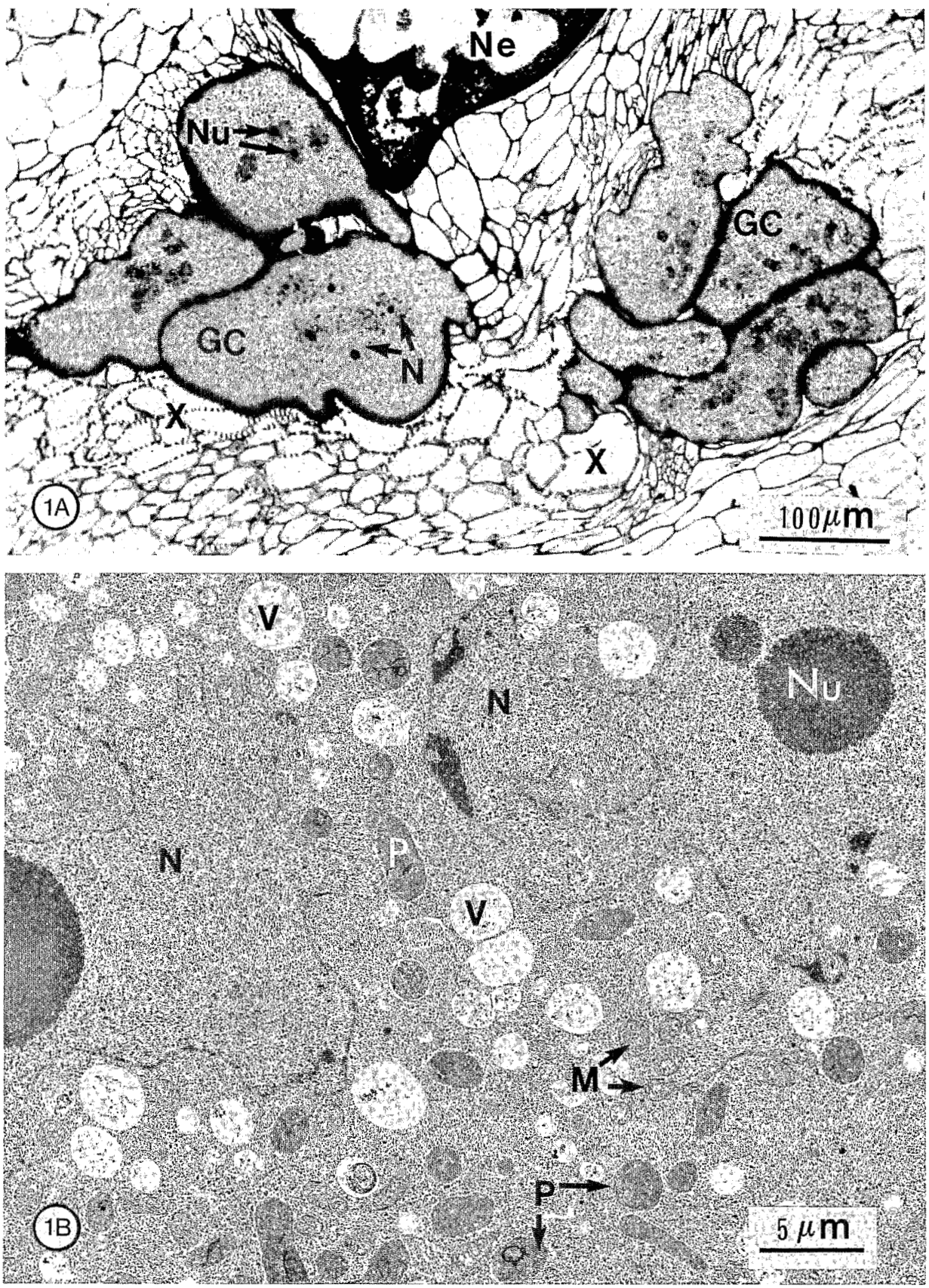

Fig. 1. Section of roots of *Lycopersicon esculentum* cultivars susceptible (A) or resistant (B) to *Fusarium*, injected with *Meloigodyne javanica* and incubated in 50 % *Fol* culture filtrate. A : After 3 h incubation, giant cells (GC) appear structurally normal (light micrograph). B : After 6 h incubation, giant cells are structurally normal. Note the well defined membranes and cellular organelles. (M = mitochondria, N = nucleus, Ne = nematode, Nu = nucleolus, P = plastid, V = vacuole, X = xylem).

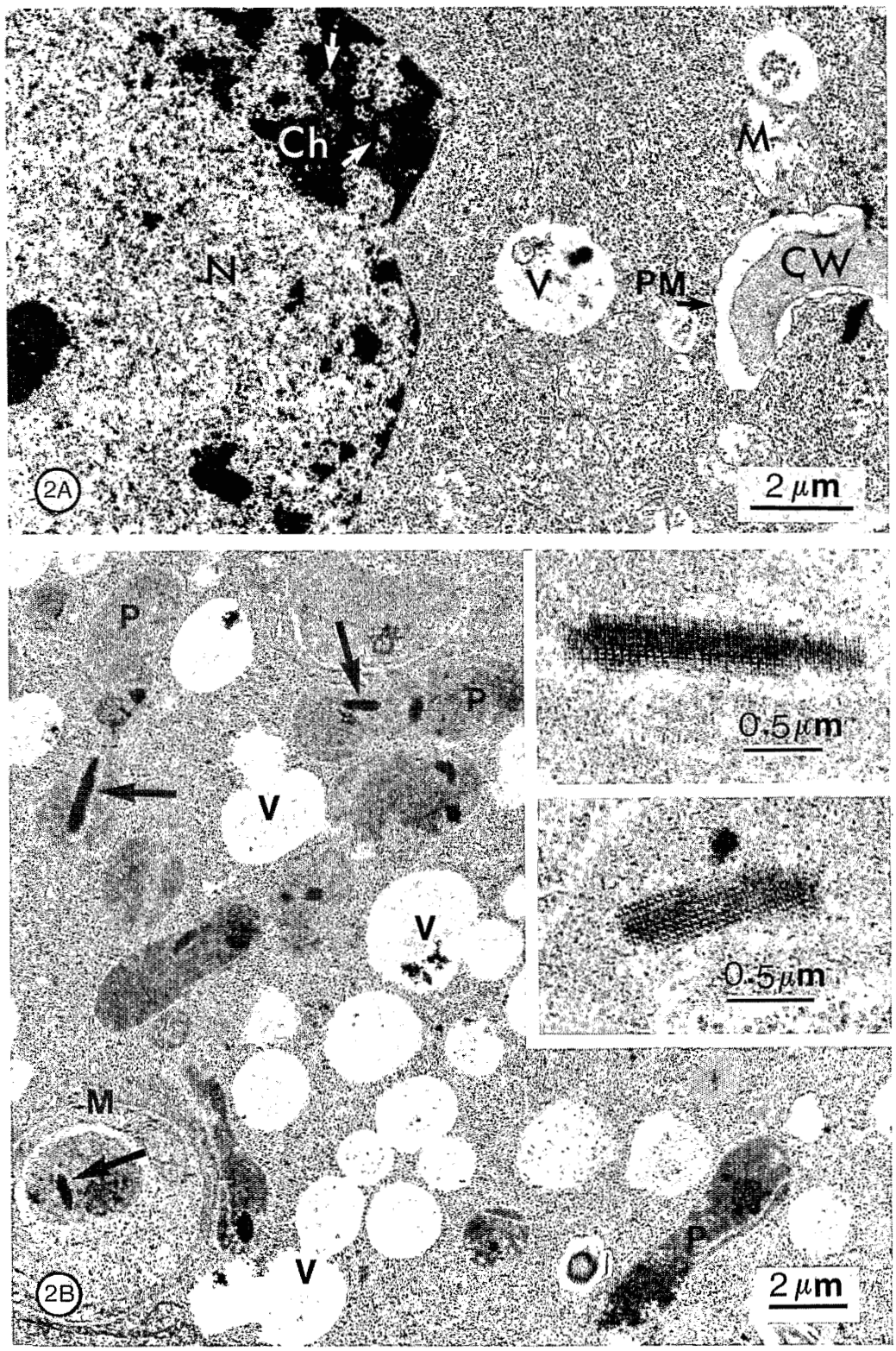

Fig. 2. Sections through giant cells induced by *Meloidogyne javanica* in *Lycopersicon esculentum* (susceptible to *Fusarium*), showing ultrastructural modifications after 12 h incubation in 50 % *Fol* culture filtrate. A : Nucleus (N) containing halo-surrounded, dark inclusions (arrows) in the circumferential accumulation of condensed chromatin (Ch.). Note the disorganized mitochondria and the electron transparent area between the cell wall (CW) and plasma membrane (PM). B : Vacuoles with degenerated tonoplast; plastids (P), with broken outer membrane, containing phytoferritin crystals (arrows). Insets are enlargement of the phytoferritin crystals. (M = mitochondrion, V = vacuole).

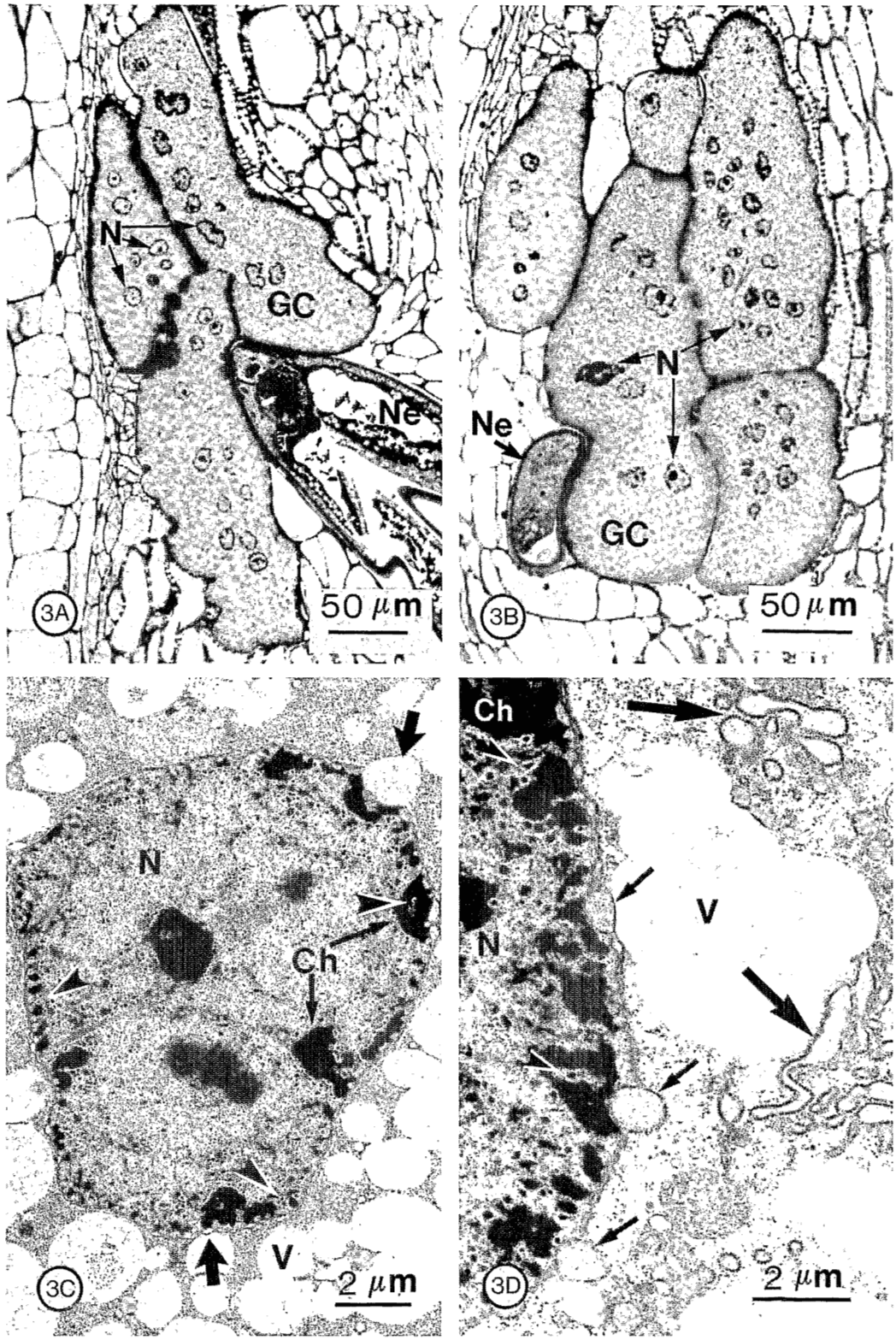

Fig. 3. Light (A & B) and electron micrographs (C & D) of sections through *Lycopersicon esculentum* (susceptible or resistant to *Fusarium*) roots, infected with *Meloidogyne javanica*, 24 h after incubation in 50 % *Fol* culture filtrate. A : Giant cells in *Fol* susceptible cultivar. Note some of the nuclei (N) are spherical with chromatin condensed along the nuclear membrane and the cytoplasm is weakly staining. B : Disrupted giant cells in *Fol* resistant cultivar. Note the nuclear chromatin condensation and weakly stained cytoplasm similar to those in A. C : Part of a giant cell in B. Spherical nucleus (N) with condensed chromatin (Ch) and dark inclusions (arrow heads). Note the swellings of the nuclear membrane (arrows). D : Part of a giant cell in A. Nucleus with condensed chromatin containing halo-surrounded dark inclusions (arrow heads), and swollen, ribosome-covered, nuclear membrane (small arrows). Note the swollen, rough, endoplasmic reticulum (large arrows). (GC = giant cell, Ne = nematode, V = vacuole).

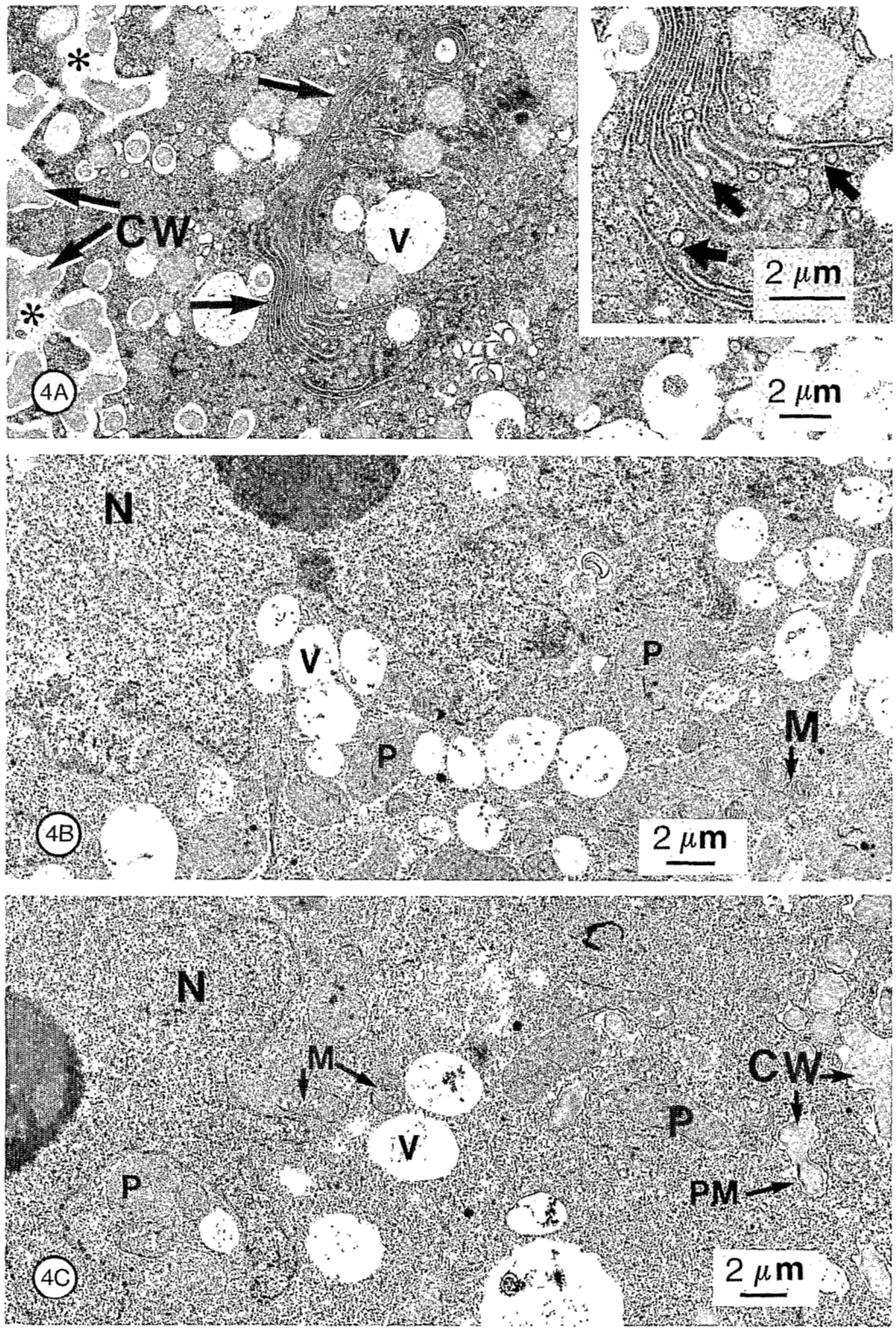

Fig. 4. A : Part of a giant cell induced by *Meloidogyne javanica* in *Fusarium* resistant tomato, incubated for 24 h in 50 % *Fol* culture filtrate. Note the parallel arrangement of rough endoplasmic reticulum (arrows) and the electron transparent zone (asterisks) between the cell wall (CW) and cytoplasm. Inset is an enlarged portion of A showing the increased number of ribosomes associated with the vesiculated endoplasmic reticulum (arrows). B : Part of a giant cell induced by *M. javanica* in *Fusarium* resistant tomato incubated for 24 h in 50 % Tochinai broth (a control) showing ultrastructurally normal features. Note the highly lobed nucleus (N). C : Structurally normal giant cell induced by *M. javanica* in *Fusarium* resistant tomato, incubated for 24 h in 50 % nutrient solution (a control). Note the distinct plasma membrane (PM). (M = mitochondrion, P = plastid, V = vacuole).

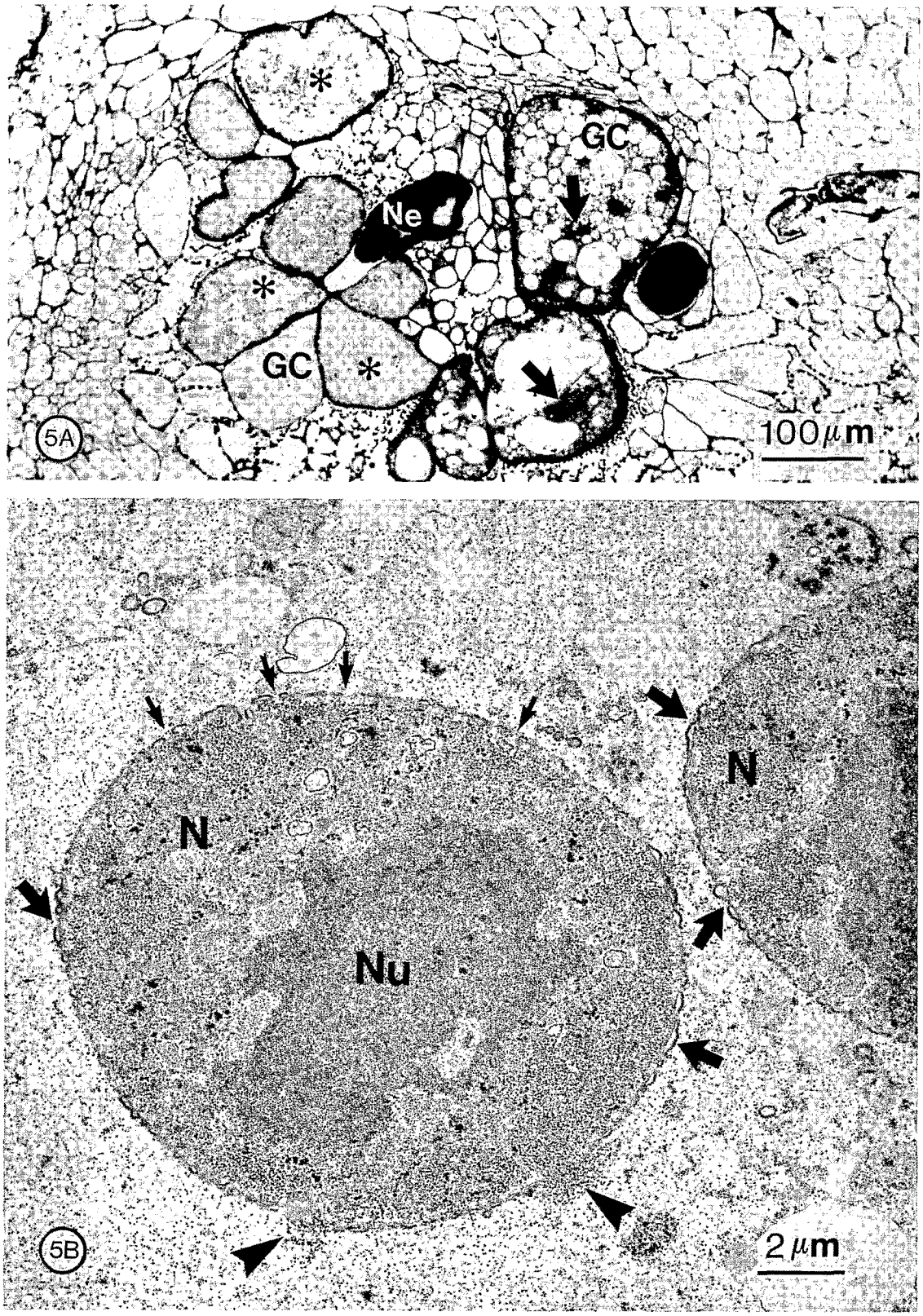

Fig. 5. Sections through roots of *Lycopersicon esculentum* resistant (A) or susceptible (B) to *Fusarium* infected with *Meloidogyne javanica* after 9 h incubation in 100 % *Fol* culture filtrate, then transferred to either water (A) or Tochinai broth (B) and incubated for a further 15 h. A : Light micrograph of root showing apparently different response of giant cells (GC) to culture filtrate treatments. Some giant cells contain lightly stained cytoplasm (asterisks), others contain densely stained, vacuolated protoplasm (arrows). B : Electron micrograph of part of disrupted giant cell. Note the spherical nuclei (N) with fragmented (small arrows), swollen and ribosome covered (large arrows) and ruptured (arrow heads) nuclear membrane. Nucleolus (Nu) is less electron dense and appears to have lost its regular outline. (Ne = nematode).

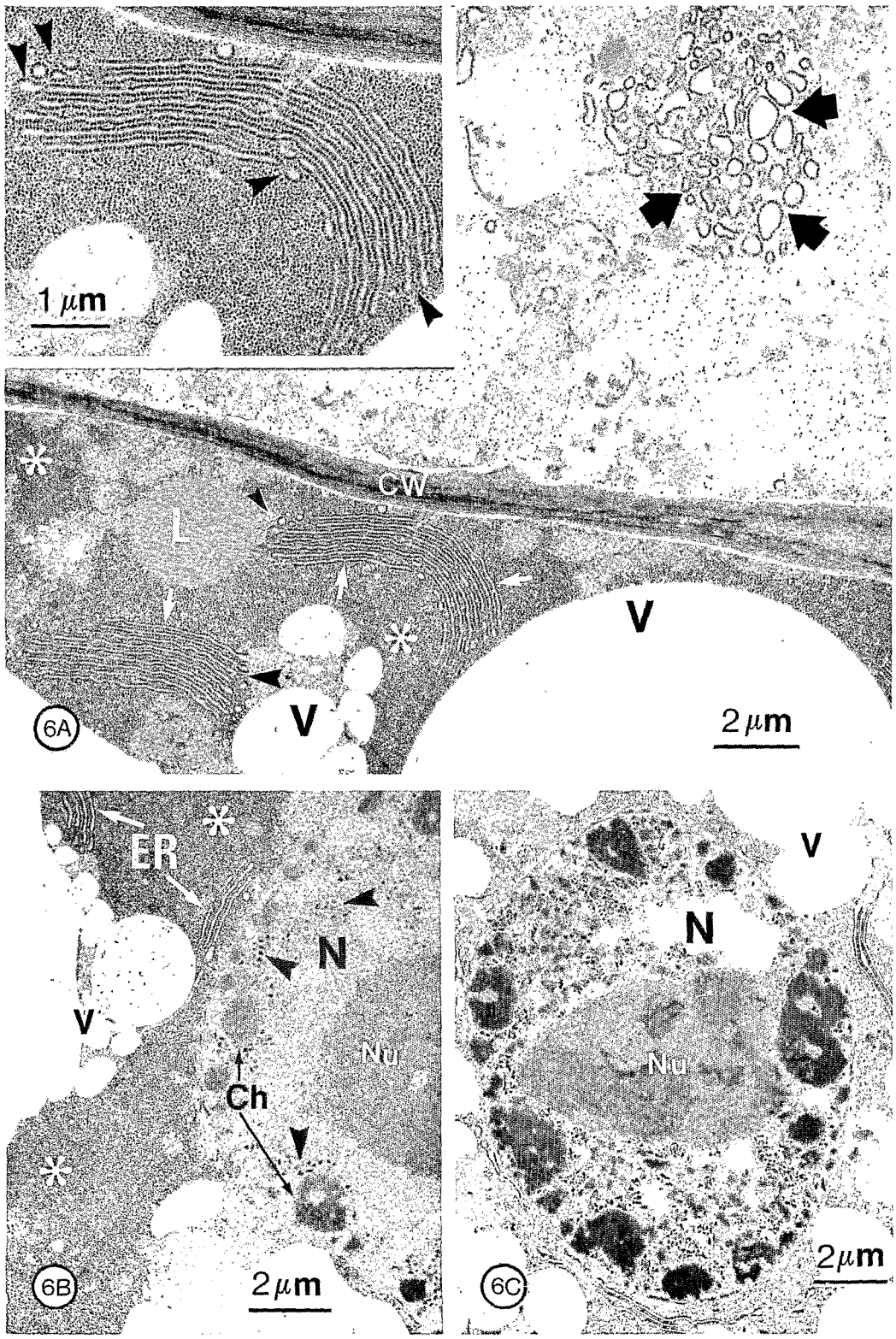

Fig. 6. *Meloidogyne javanica* infected *Lycopersicon esculentum* roots resistant (A & B) or susceptible (C) to *Fusarium*, after 9 h incubation in 100 % *Fol* culture filtrate, then transferred to Tochinai broth and incubated for a further 15 h. A : Part of two adjacent cells showing different response to culture filtrate treatment. Top cell with electron light cytoplasm containing remnants of cell organelles and swollen, rough endoplasmic reticulum (large arrows). Lower cell contains electron dense cytoplasm with a large number of ribosomes (asterisks), parallel stacks of rough, vesiculated endoplasmic reticulum (arrow heads and enlarged inset). Note the apparent lack of plasma membrane in both cells. B : Modified nucleus (N) with condensed chromatin (Ch) and dark inclusions (arrow heads). Note the abnormal rough endoplasmic reticulum (ER) and the large number of ribosomes (asterisks). C : Modified nucleus with condensed chromatin. Note the abnormal nucleolus (Nu) with irregular outlines. Note the cytoplasm is less electron dense than that in B. (CW = cell wall, L = lipid, V = vacuole).

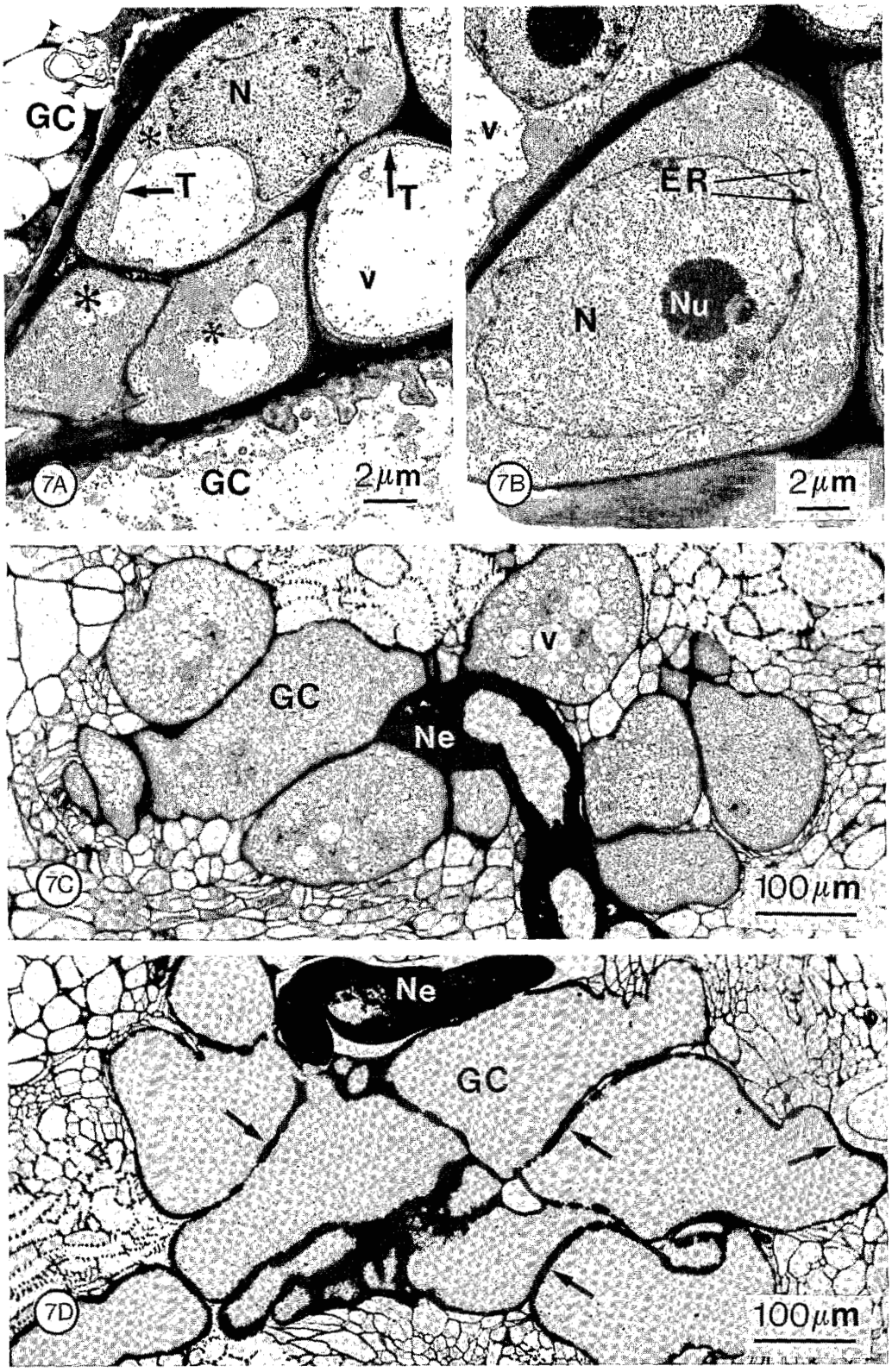

Fig. 7. A and B : Sections through *Meloidogyne javanica* infected roots of *Lycopersicon esculentum* (susceptible to *Fusarium*) after 9 h incubation in 100 % *Fol* culture filtrate, then transferred to Tochinai broth and incubated for a further 15 h. A : Parenchyma cells (asterisks) adjacent to deteriorated, electron light giant cells (GC) in a section near the root tip. Note the normal looking nucleus and well preserved tonoplast (T) in the parenchyma cells. B : Parenchyma cell showing ultrastructurally normal cellular organelles. C and D : Giant cells induced by *M. javanica* in *Fol* resistant *L. esculentum* incubated in *Fol* culture filtrate for 24 h (D) or for 30 min, then transferred to Tochinai broth and further incubated for 24 h (C). C : Showing structurally normal giant cells. D : Deteriorated giant cells containing remnants of cell organelles. Note the normal cell walls (arrows). (ER = endoplasmic reticulum, N = nucleus, Ne = nematode, Nu = nucleolus, V = vacuole).

the light microscope nuclear and cytoplasmic disruption was visible in the giant cells, but the cell walls appeared unaffected by any of the filtrates even after 24 h incubation in 100 % culture filtrate. In this experiment root samples were collected five weeks after nematode inoculation, and so it was possible to observe the effects of fungal culture filtrates on mature giant cells (associated with adult females) and young giant cells (associated with juveniles). Young giant cells appeared to be more sensitive to fungal filtrate treatment than mature giant cells because there was extensive degeneration of the contents of young compared with mature giant cells. Parenchyma cells around the giant cells of filtrate treated and control roots appeared normal.

Incubation in phosphate buffer (pH 8.3) caused minor ultrastructural changes (e.g. changes in electron density of the cytoplasm and nucleoplasm) to the giant cells although the plasma membrane and tonoplast appeared to be unaffected. These ultrastructural changes were dissimilar to those caused by fungal filtrates. The ultrastructure of giant cells in roots incubated in phosphate buffer (pH 6.8) was similar to that of giant cells in the controls.

Neither the pH nor the heat treatments of the *Fol* culture filtrate modified its effect on the giant cells of the *Fol* resistant tomato cultivar.

After 24 h incubation in *Fol* culture filtrate (100, 50 and 25 %) all *M. javanica* juveniles were mobile, and were structurally indistinguishable from those in the roots of control treatments. The treated juveniles infected young tomato seedlings and induced the formation of ultrastructurally " normal " giant cells.

Discussion

This ultrastructural study supports the concept that translocatable toxic metabolites from plant pathogenic fungi cause deterioration of *M. javanica* induced giant cells. Cell-free, *Fol* culture filtrate elicited ultrastructural modifications of *M. javanica* induced giant cells in *Fol* resistant and susceptible tomato cultivars. These modifications were indistinguishable from those of giant cells in tomato plants infected with *Fol* (Fattah & Webster, 1983). The absence of fungus in many of the modified giant cells of such fungus-infected plants and in those treated with the cell-free culture filtrate suggests that these ultrastructural changes are caused by translocatable, toxic metabolite(s) produced by the fungus in either the plant host or the culture medium.

After 24 h incubation of nematode infected, *Fol* resistant and susceptible tomato roots in *Fol* culture filtrate, ultrastructural changes occurring in the giant cells of the two cultivars were almost identical (Figs 3-5). However, Fattah and Webster (1983) showed that the changes in *Fusarium* infected plants occurred earlier in susceptible than in resistant cultivars. This implies that the nematode does not completely break the fungal resistance of the Pearson Improved tomato cultivar. This supports the view that *Fusarium* resistance in tomato (Sidhu & Webster, 1974) is influenced by several factors, only some of which are modified by nematode infection. The delay in giant cell deterioration after *Fol* infection in *Fol* resistant tomato may be due to delayed hyphal development and associated lower production of fungal metabolites in the *Fusarium* resistant cultivar as compared with that in the susceptible cultivar.

At least three factors influence the ultrastructural modifications of giant cells when treated with *Fol* culture filtrate, namely concentration of the culture filtrate, duration of exposure of nematode infected tissues to the culture filtrate and the time required to express ultrastructural modifications. Incubation of nematode- infected roots for 9 h in 100 % *Fol* culture filtrate produced ultrastructural changes in giant cells similar in form and severity to those produced by incubation of nematode-infected roots for 24 h in 50 % *Fol* culture filtrate. Furthermore, no ultrastructural changes were detected after incubation in 50 % culture filtrate for 6 h or 100 % for less than 3 h. This suggests that the accumulation of toxic substances within the giant cells was insufficient to produce ultrastructural changes. Alternatively, for this initial period, the giant cells are capable of neutralizing the localised phytotoxic effects of the culture filtrate. Deterioration of giant cells of the same physiological age when exposed to the filtrate appears to be directly correlated with the concentration of the filtrate. Evidence for this is that almost complete degeneration of giant cell contents occurred when excised nematode infected roots were incubated in 100 % fungal culture filtrate (Fig. 7 D) whereas some cellular organelles in giant cells were retained when incubated for the same period in 50 % filtrates (Fig. 3 A, B).

Exposure to 100 % fungal culture filtrate caused significant structural modification of giant cells by 9 h. These modifications were not reversible by subsequent 15 h incubation in either Tochinai broth medium or in water. From this study it is impossible to determine the precise sequence of the ultrastructural response in giant cells caused by the fungal metabolites despite the relative synchrony of the experiments. It is possible that the initial increased electron density of cell cytoplasm and an associated increase in metabolic response was followed by an ultrastructural and physiological collapse associated with decreased cytoplasmic electron density as the cellular organelles broke down (see Fig. 6 A).

Fungal culture filtrate treatment and fungal infection (Fattah & Webster, 1983) of nematode infected tissues apparently causes degeneration of cellular membranes. The plastid membrane and the tonoplast were the first structures to degenerate in *Fol* culture filtrate treatment (Fig. 2 A, B). Degeneration of the plastid outer membrane, tonoplast and plasma membrane in fungal culture filtrate treated giant cells parallels the response

of leaf cells of *Beta vulgaris* treated with cercosporin (Steinkamp *et al.*, 1981).

Young giant cells were more sensitive to fungal culture filtrate than mature giant cells. Young and mature giant cells have different relative rates of metabolism, and previous studies with phytotoxins have shown that the sensitivity of plant tissues to toxins varies with plant age and activity. Oat seedlings are increasingly sensitive to victorin produced by *Helminthosporium victoriae* up to 18 d after germination (Damann, Gardner & Scheffer, 1974), and young broad bean leaves are markedly more sensitive than old leaves to *Spiroplasma citri* toxin (Daniels, 1979).

The increased cytoplasmic density of giant cells in root tissues treated with either 50 % fungal culture filtrate for 24 h (Fig. 4 A) or 100 % fungal culture filtrate for 9 h (Fig. 6 A, B) suggests a correlation of fungal culture filtrate concentration and time of incubation with giant cell metabolic stimulation prior to death. The increased cytoplasmic density, due to the unusually large number of ribosomes either free or bound to the endoplasmic reticulum, in indicative of a higher metabolic activity. The giant cells may, therefore, be responding to the initial accumulation of toxic metabolites by increasing synthesis of protein precursors for enzyme synthesis or for structural proteins in the initial repair response. Prolonged exposure to fungal culture filtrate results in degeneration of cellular organelles and permanent damage to the giant cells. Association of ribosomes with the nuclear membrane and the occurrence of dense granules in nuclei also occurs in giant cells of *Fol* infected plants (Fattah & Webster, 1983). In the early stages of infection by vascular wilt fungi, the cytoplasm aggregates in regions of the cell adjacent to extracellular hyphae. These regions show increased numbers of ribosomes and amounts of rough endoplasmic reticulum (Bishop & Cooper, 1983). This phenomenon has been reported in other plant host-parasite interactions including the initial resistance response of tomato to *M. incognita* (Paulson & Webster, 1972; Mercer, Wood & Greenwood, 1975).

None of the treatments with fungal culture filtrates caused detectable structural modifications of giant cell walls (Figs 3 A , B; 7 D). This may be due to the limited incubation time of nematode infected roots in the culture filtrates or absence of appropriate fungal enzyme secretions in the culture medium. Furthermore, heated *Fol* culture filtrate produced ultrastructural modifications of giant cells similar to those resulting from treatments with unheated culture filtrate. This implies that filtrate toxicity was not due to enzymes.

Nematode tissue in sections from fungal culture filtrate treatments was ultrastructurally normal. Furthermore, incubation of infective, juvenile *M. javanica* in *Fol* culture filtrate for 24 h did not affect juvenile viability, infectivity or ability to induce " normal " giant cells. In contrast, the culture filtrates of *Sclerotium rolfsii* (Shukla & Swarup, 1971), *Helminthosporium nodulosum, Trichoderma lignorum, Curvularia tuberculata, Penicillium corylophium* and *Aspergillus niger* have been shown to be toxic to *M. incognita* juveniles (Alam, Khan & Saxena, 1973). The difference between our results and those reported earlier by Shukla and Swarup (1971) and by Alam, Khan and Saxena (1973) may be due to the use of different species of fungi and/or to the experimental conditions. Endomycorrhizal *(Glomus mosseae)* infected tomato plants retarded giant cell formation when subsequently infected with *M. incognita*, but the fungus was not observed in the giant cells (Sikora, 1979). *F. oxysporum* is pathogenic to alfalfa but does not alter population size of *Pratylenchus penetrans.* In contrast, *F. solani* is not pathogenic to alfalfa and decreases the *P. penetrans* population (Mauza & Webster, 1982). *Fusarium* wilt fungi appear to enhance reproduction of the migratory nematode *Tylenchorhynchus claytoni* on pea roots (Davis & Jenkins, 1963). Culture filtrates from *Fol* and *Cc* pathogenic to tomato, and from *Fsp* non pathogenic to tomato, caused similar giant cell deterioration. It is not known whether giant cell deterioration is caused by toxic metabolite(s) common to all these pathogens or is the result of species specific toxic metabolite(s).

The ultrastructural modifications of giant cells in the present study were not caused by the pH of the fungal culture filtrates, and those that occurred were indistinguishable from those caused by *Fol* infection of tomato. The retraction of cytoplasm in giant cells treated with fungal culture filtrate (Figs 2 A, 4 A) and in giant cells in *Fol* infected tomato plants (Fattah & Webster, 1983) could be the result of " false plasmolysis ". False plasmolysis has been reported for cells of fungal infected plants (Kenning & Hanchey, 1980) and in plants treated with fungal toxic metabolites (Hanchey & Wheeler, 1969; Hawes, 1983). The retraction of cytoplasm from the cell wall in giant cells cannot be explained as normal plasmolysis because the osmolarity of the Tochinai broth (100 % filtrate = 89 and 50 % filtrate = 75 milliosmoles/kg) was higher than that of the fungal culture filtrates (100 % = 77 and 50 % = 60 milliosmoles/kg), and giant cells incubated in Tochinai broth showed no such modifications.

Parenchyma cells adjacent to giant cell clusters appeared to be less sensitive to fungal culture filtrate treatments than giant cells because degeneration of giant cells occurs before it does in parenchyma cells (Fig. 7 A, B). Hawes (1983) showed a differential response between root hairs and leaf mesophyll cells which are more sensitive to victorin treatment than are root cap cells. As speculated by Orion, Wergin and Endo (1980), the factors affecting root growth appear to be independent of those affecting giant cell initiation and development.

The differential sensitivity of giant cells and parenchyma cells to fungal culture filtrate suggests a potential for the use of fungal toxic metabolites to selectively

disrupt giant cells without concommitant significant harmful effects on normal plant cells. However, phytotoxicity of culture filtrates from several plant pathogenic fungi has been demonstrated (Gilchrist & Grogan, 1976; Strange, Pippard & Strobel, 1982). Prevention of giant cell formation by the application of plant growth hormones (Sawhney & Webster, 1975), plant growth inhibitors (Davide & Triantaphyllou, 1968; Kochba & Samish, 1972; Orion & Minz, 1971), and antibiotics (Orion, 1973) severely damaged the host plant and, consequently, limited their use as control agents for the root-knot nematode.

The occurrence of crystals within the plastids of giant cells in fungal filtrate treated root tissues and *Fol* infected tomato plants could be due to physiological stress caused by toxic metabolites from the fungus. Phytoferritin crystals have been reported in the plastids of senescent leaves (Ljubesic, 1976), of iron-starved sugar beet, *Beta vulgaris* leaves, and of chlorotic leaves of *Mycoplasma* infected coconut palm, *Cocos nucifera* L. (Hunt, Dabek & Schuiling, 1974).

Although this is the first study to describe ultrastructural modifications of the giant cells by *Fol* fungal culture filtrate treatments, they are somewhat similar to the modifications of plant cells caused by other pathogens. Nuclei in the cells of cercosporin treated leaves of *B. vulgaris* showed peripheral condensation of chromatin (Steinkamp *et al.*, 1981), and nuclear membrane swelling and rupture was observed in the cells of rust infected wheat, *Triticum compactum* L. (Manocha & Shaw, 1966). These ultrastructural modifications are very similar to those in giant cells after fungal culture filtrate treatment (Figs 3 C, D; 5 B) and *Fol* infection of tomato plants (Fattah & Webster, 1983). Swellings and vesiculation of the endoplasmic reticulum occur in the host cells of pea, *P. sativum*, and soybean, *Glycine max*, after rhizobial endocytosis (Kijne & Planque, 1979) and in resistant tomato infected with *Cladosporium fulvum* (Lazarovits & Higgins, 1979). Comparable modifications of the endoplasmic reticulum are produced by abiotic factors such as ethylene (Freytag, Berlin & Linden, 1977), flooding (Pomeroy & Andrews, 1979) and anaerobiosis (Whaley, Kephart & Mollenhauer, 1964). Hence, diverse chemicals or physiological factors produce similar ultrastructural modifications of plant cells.

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