Ultrastructural Changes Caused by Fusarium oxysporum f. sp. lycopersici in Meloidogyne javanica
Induced Giant Cells in Fusarium Resistant and Susceptible Tomato Cultivars

F. Fattah and J. M. Webster

Abstract: Tomato (Lycopersicon esculentum Mill.) seedlings, susceptible (cv. Pearson A-1 Improved) and resistant (cv. Pearson Improved) to race 1 Fusarium oxysporum f. sp. lycopersici (Sacc.) Syd & Hans., were inoculated with Meloidogyne javanica (Trueb) Chitwood second-stage juveniles and 3 weeks later with race 1 F. oxysporum f. sp. lycopersici spores. One week after fungal inoculation, no fungus was visible in root tissue of the tomato cultivars and the giant cells were normal. Two weeks after fungal inoculation, abundant hyphae were visible in xylem tissues of Fusarium-susceptible but not of Fusarium-resistant plants. In susceptible plants, giant cell degeneration occurred, characterized by membrane and organelle disruption. In addition, where hyphae were in direct contact with the giant cell, dissolution of the giant cell wall occurred. Three weeks after fungal inoculation, fungal hyphae and spores were visible inside xylem tissues and giant cells in Fusarium-susceptible plants and in xylem tissue of the resistant plants. In susceptible and resistant plants, giant cell degeneration was apparent. Giant cell walls were completely broken down in Fusarium-susceptible tomato plants. In both cultivars infected by Fusarium, giant cell nuclei became spherical and dark inclusions occurred within the chromatin material which condensed adjacent to the fragmented nuclear membrane. No such ultrastructural changes were seen in the giant cells of control plants inoculated with nematode alone. Giant cell deterioration in both cultivars is probably caused by toxic fungal metabolites.

Key words: root-knot nematode, Fusarium oxysporum f. sp. lycopersici, histopathology, giant cell, ultrastructure, disease complex.


The root-knot nematode–Fusarium wilt interaction on tomatoes was first observed by Young (22), who suggested that the nematode infection diminished resistance of tomatoes to Fusarium wilt. The extent and mechanisms of this predisposition of plants by nematodes to fungal infection has been the subject of many investigations (4,8,9,18,19) and reviews (1,15,17). Fungal colonization of giant cells and xylem tissue has been described for cotton roots simultaneously infected with F. oxysporum f.sp. vasinfectum and Meloidogyne incognita (9) and for tobacco infected with M. incognita acrita and Phytophthora parasitica var. nicotianae (18). Melendez and Powell (8) showed that extensive fungal invasion of giant cells resulted in loss of cytoplasmic contents; they regarded this as evidence of sensitivity of the giant cells to fungal invasion.

All reported histological studies of this fungal-nematode disease complex have used light microscopy. This ultrastructural study was undertaken on fungal-resistant and susceptible hosts to better elucidate the intracellular sequence of events at the organelle level and to show the relative importance of hyphal penetration and fungal toxins on the deterioration of giant cells.

Received for publication 7 June 1982.

1Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, B.C. V5A 1S6, Canada.

Figs. 1–4. Meloidogyne javanica induced giant cells in the roots of tomato (Lycopersicon esculentum), susceptible (Pearson A-1 Improved), or resistant (Pearson Improved) to race 1 Fusarium oxysporum f. sp. lycopersici Syd. & Hans., at various times after fungal inoculation. 1) Resistant plant 1 week after fungal inoculation. Note the giant cells with the characteristic thick cell walls, dense multinucleate cytoplasm, and lobed nuclei with prominent nucleoli. (GC = giant cell, N = nucleus, Nu = nucleolus, X = xylem.) 2) Susceptible plant 1 week after fungal inoculation. Note the highly lobed nucleus (N) with prominent nucleolus (Nu). (GC = giant cell, M = mitochondrion, P = plastid, v = vacuole.) 3) Susceptible plant 2 weeks after Fusarium inoculation. Note fungal hyphae and spores (F) in the xylem vessels (X) and the initial breakdown of the giant cells with nuclear and cytoplasmic changes. (GC = giant cell, N = nucleus.) 4) Electron micrograph of affected giant cell (GC) in Fusarium susceptible plant 2 weeks after fungal inoculation showing electron dense inclusions (arrow heads) within the chromatin material (Ch) and the fragmented (small arrows), swollen and ribosomes covered (large arrows) nuclear membrane. (N = nucleus.)
MATERIALS AND METHODS

Tomato (Lycopersicon esculentum Mill.) seedlings cv. Pearson A-1 Improved, susceptible to race 1 Fusarium oxysporum f.sp. lycopersici (Sacc.) Snyd. & Hans., and cv. Pearson Improved, resistant to this Fusarium race, were grown in small plastic pots of vermiculite. The seeds were surface sterilized with 0.5% mercuric chloride in acidified 70% ethanol and then thoroughly washed prior to planting. Plants were maintained in a growth chamber with 28 ± 1°C day and 20 ± 1°C night temperatures and a 16-hour photoperiod with a light intensity of 150 µE m⁻² sec⁻¹ from three cool white, fluorescent (General Electric, USA) and one Grow-Lux (Westinghouse, Canada) lamps.

Second-stage juveniles of Meloidogyne javanica (Trueb) Chitwood were hatched from egg masses collected from stock cultures on the roots of lima bean (Phaseolus lunatus L. var. L-136) and surface sterilized for 10 min in 0.1% (p-chlorophenyl-diguanido) hexane diacetate (Hibitane) (13). An aqueous suspension of approximately 2,000 freshly hatched, surface sterilized, second-stage juveniles of M. javanica was poured around each potted, 20-day-old tomato seedling. Three weeks later, a 10-ml aqueous spore suspension containing 2 × 10⁶ Fusarium spores/ml was poured in three holes in the soil adjacent to each tomato seedling. Plants were watered on alternate days with water and nutrient solution (10).

Specimens of root for light and electron microscope examination were taken from infected and control plants at weekly intervals for 3 weeks after fungal inoculation. Pieces of root were fixed in 30% glutaraldehyde in 0.025 M phosphate buffer, pH 6.8, and post fixed in 2% osmium tetroxide in the same buffer (11), dehydrated in a graded acetone series and propylene oxide and then infiltrated with low viscosity epoxy resin (20). Sections for light microscopy were cut at 0.5–1 µm thickness, using a glass knife, and stained with 1% toluidine blue 0. Silver-grey sections were cut with a diamond knife for electron microscopy and stained with aqueous uranyl acetate (10 min) and lead citrate (5 min) for viewing in a Phillips EM 300 electron microscope.

RESULTS

One week: No vascular wilt symptoms or cellular evidence of fungal invasion was observed in roots of either Fusarium-susceptible or resistant tomato cultivars. Giant cells in the roots of both cultivars appeared typical in that membranes and cellular organelles were intact (Figs. 1, 2). Giant cells in roots of both cultivars inoculated with the fungus were structurally similar to those in the roots of the fungus-free controls.

Two weeks: Abundant fungal growth was detected in the xylem tissue of susceptible plants especially in xylem vessels adjacent to the giant cells (Fig. 3). Although no fungal hyphae were detected inside giant cells of Fusarium-susceptible plants, the giant cells often showed membrane breakdown and disruption of cell organelles (Figs. 3, 4). In these giant cells, the cytoplasm appeared electron light, the giant cell nuclei showed electron dense inclusions within the chromatin material, and the nuclear membrane was partially fragmented, swollen, and covered with ribosomes (Fig. 4). Giant cells in Fusarium-resistant roots were unaffected by the fungus and resembled the giant cells in the fungus-free controls.

Figs. 5-8. Meloidogyne javanica-induced giant cells in the roots of tomato (Lycopersicon esculentum Mill.), susceptible ('Pearson A-1 Improved') or resistant ('Pearson Improved') to race 1 Fusarium oxysporum f. sp. lycopersici Snyd. & Hans., at various times after fungal inoculation. 5) Fusarium hypha (F) within the cell wall (CW) between two giant cells in the susceptible cultivar 2 weeks after fungal inoculation. Note the cell wall dissolution (astisk) adjacent to the fungal hypha. 6) Disrupted giant cell (GC) in Fusarium-resistant cultivar with nuclear and cytoplasmic changes 3 weeks after fungal inoculation. Note fungal hyphae and spores (F) in xylem vessels (X). (N = nucleus.) 7) Breakdown of cell walls and contents in giant cell clusters (GCC) and parenchyma cells in Fusarium susceptible tomato 3 weeks after fungal inoculation. Note Fusarium hyphae and spores (arrows) within the giant cell clusters and xylem vessels (X). 8) Electron micrograph of Fusarium (F) in the giant cell (GC) of fungus susceptible cultivar 5 weeks after fungal inoculation.
the Fusarium-susceptible cultivar where fungal hyphae were in direct contact with the giant cell wall, dissolution of the cell wall was evident as an electron light area and by the absence of cellulose fibers close to the fungal hyphae (Fig. 5).

Three weeks: Fungal hyphae and spores were visible in xylem tissue of the Fusarium-resistant (Fig. 6) and susceptible cultivars and inside giant cells of the susceptible cultivar (Fig. 7). At this stage of fungal invasion, the giant cell walls and some adjacent parenchyma cell walls in the fungus susceptible tomato were severely broken down resulting in a large cavity containing cell debris, fungal hyphae, and spores (Figs. 7, 8).

By the end of 3 weeks, the giant cell nuclei in both cultivars infected with Fusarium were smaller, spherical (Figs. 3, 9), and had lost the irregular shape characteristic of nematode-induced giant cells in fungus-free plants (Figs. 1, 2). Nuclei in fungus-affected giant cells of both cultivars had a large central light area and chromatin material condensed along the nuclear membrane (Figs. 3, 9). The nuclear membrane appeared partially fragmented, swollen (Figs. 4, 9, 10), and covered with ribosomes (Figs. 4, 10). Dark inclusions with an electron-light halo occurred within the peripheral nuclear chromatin material of Fusarium-affected giant cell nuclei (Figs. 4, 9, 10). Ribosomes were the most recognizable cytoplasmic organelles in fungus-affected giant cells of both cultivars; they occurred throughout the cytoplasm and attached to the nuclear membrane (Figs. 4, 10) and to membrane-bound spherical bodies (Fig. 11).

Crystalline inclusions occurred within the plastids of Fusarium-affected giant cells in both fungus-resistant (Fig. 11) and susceptible cultivars. The giant cells in both cultivars inoculated with Meloidogyne alone and in those fungus-inoculated plants that escaped the fungal infection appeared normal even 3–6 weeks after nematode infection, as manifest by well-defined membranes, large highly lobed nuclei, and dense cytoplasm.

DISCUSSION

These results confirm earlier light microscope observations (8,18) on the affect of the fungus on nematode-induced giant cells in this root-knot–wilt disease complex. However, electron microscopy has facilitated a better understanding of the temporal sequence of intracellular changes when comparing the response of Fusarium-resistant and susceptible cultivars in relation to the proximity of fungal hyphae.

The apparent absence of Fusarium within the root tissues of either susceptible or resistant tomato cultivars 1 week after fungus inoculation probably is due to the relatively slow rate of spore germination and/or hyphal invasion and to the fact that fungal invasion was so limited as to be undetected at the time of sampling. Nevertheless, this delay in detecting fungal hyphae in the roots parallels that reported by Melendez and Powell (8) for F. oxysporum f. nico- tianae in tobacco infected with M. incognita. Progressive invasion of the xylem vessels, xylem parenchyma, and giant cells in the susceptible cultivar by the Fusarium hyphae was observed after the first week and this culminated in breakdown of the giant cells about 3 weeks after fungal inoculation. Earlier light microscope observations (8) did not show any significant intracellular changes in the giant cells until after their invasion by fungal hyphae. However, the present study reveals in the fungus-susceptible (Figs. 3, 4) and fungus-resistant (Figs. 6, 9, 10) cultivars considerable ultrastructural changes in giant cells prior to

Figs. 9–11. Meloidogyne javanica induced giant cells in the roots of tomato (Lycopersicon esculentum Mill. cv. Pearson Improved), resistant to race 1 Fusarium oxysporum f.sp. lycopersici Snyd. & Hans., 3 weeks after fungal inoculation. 9) Nuclei (N) with electron light central area showing fragmented nuclear membrane (large arrows) and dark inclusions (small arrows) within the chromatin material (Ch). (Ch = chromatin.) 10) Electron micrograph showing nucleus (N) with ribosomes attached to the nuclear membrane (large arrows) and nuclear membrane swellings (small arrows). (Ch = chromatin.) 11) Fusarium-affected giant cell (GC) with crystalline inclusions (large arrow) and ribosomes attached to membrane bound spherical bodies (short arrow). Insert showing the crystalline inclusions (large arrow) within plastid (P). (L = lipid, M = mitochondrion.)
their invasion by fungal hyphae. This is not unexpected in view of the known secretions of *Fusarium* wilt fungi (14, 21); e.g., hydrolyzing enzymes, fusaric acid, and lycomarasmine. Rapid deterioration of giant cells in *Fusarium*-infected plants is likely initiated by toxic metabolites from the fungus outside the cell, as distinct from the process of hyphal invasion and growth within the cell, because a) giant cell deterioration occurred without corresponding hyphal penetration, b) giant cell deterioration in fungus infected plants (Figs. 3, 4) is a dissimilar process to that of natural aging of giant cells after female maturation (11), and c) the developing nematode appeared to be fungus free. This early recognition of disrupted giant cell protoplasmic contents in both cultivars, even when no hyphae were visible within the giant cells, suggests that giant cells are sensitive to a translocatable physiological factor. This was not reported in earlier papers on the histopathology of the *M. incognita*-*Fusarium* (8,9) and *M. incognita* acrita-*P. parasitica* disease complexes (18).

Although there were abundant fungal hyphae within the xylem tissue of both cultivars 3 weeks after fungal inoculation, the hyphae occurred within the giant cells of only the *Fusarium*-susceptible plants, which confirms earlier light microscope observations (8,18). The early presence of fungal hyphae within the tissues but not in giant cells of the *Fusarium*-resistant cultivar after 3 weeks, even though they eventually enter and break down the giant cell, implies that in predisposing the plant to fungal infection the giant cell initiates a factor for fungal development, but that the giant cell itself is penetrated by the hyphae only after a threshold of infection is reached.

Powell and Nusbaum (18) described the cytoplasm in *M. incognita* acrita-induced giant cells in tobacco as becoming dense and granular during *P. parasitica* var. *nicotianae* invasion. This light microscope observation parallels the electron microscope observation of cytoplasm containing many ribosomes, ribosome covered bodies, and an abundance of crystalline structures within plastids (Fig. 11). Furthermore, giant cell degeneration culminated in a shrivelling (light microscope) (18) or a loss (electron microscope) of cytoplasmic contents. This type of giant cell breakdown contrasts with that of the normal giant cell aging process after nematode maturation; in this latter process a major feature is the progressive loss of nuclear chromatin and of membrane distinctness (11).

Crystalline inclusions within the plastids of *Fusarium*-affected giant cells may indicate a physiological stress induced by *Fusarium* toxic metabolites. These crystalline inclusions (Fig. 11) differ from those in giant cells induced by *M. incognita* alone in tomatoes (11) both in their location and structure. Crystalline inclusions within plastids in nurse cells induced by the citrus nematode, *Tylenchulus semipenetrans*, were reported (7) to be caused by “physiological disturbances” induced by nematode parasitism. However, curiously similar crystalline inclusions, composed of phytoferritin, occur in the cotyledon of *Vicia faba* during maturation (5). The ultrastructural changes of giant cells in fungus-infected plants—namely, condensation of the chromatin material peripherally in the nucleus, presence of electron dense inclusions within the chromatin material, and the association of ribosomes with the fragmented nuclear membrane (Fig. 4)—suggest major physiological and biochemical disturbances in the giant cell. Somewhat similar ultrastructural changes occur in the root cells of the nematode-resistant tomato cv. Nematex (12) when attacked by *M. incognita*. In both these examples (7,12), a physiological stress is exerted on the plant cells. Fragmentation of the nuclear membrane was reported also as a cell response in tobacco infected by *P. parasitica* var. *nicotianae* (6).

Even after 3 weeks, fungal hyphae were not observed within giant cells of the fungal-resistant cultivar, although the influence of adjacent hyphae modified giant cell ultrastructure (Figs. 9–11). Furthermore, it takes about 3 weeks for the fungus to initiate intracellular changes within giant cells of the resistant cultivar but only 2 weeks in the susceptible one. Undoubtedly, the sequence of events when *Fusarium* eventually invades giant cells of the *Fusarium* resistant cultivar is similar to that in the susceptible cultivar, except that it takes longer.
Giant cells induced in tomato by *M. javanica* are at their metabolically most active stage, and contain maximum concentrations of DNA (2) and photosynthates (3), about 3–4 weeks after the induction. It is also at this stage that *Meloidogyne* has its greatest capability for predisposing plants to *Fusarium* (16). The importance, therefore, of such a food source in enhancing *Fusarium* development, especially in a fungal resistant plant, further explains the predisposition phenomenon (17,18,19). Furthermore, the successful initiation and maintenance of giant cells is essential for normal growth and reproduction of the root-knot nematode. In fungal-resistant cultivars, this facilitates predisposition of plants to *Fusarium* wilt fungus, the toxic metabolites of which may initiate early breakdown of the giant cells. If sufficient numbers of the giant cells clustered around the head of a female nematode are destroyed, the nematode will die prematurely and so affect the overall root-knot nematode population. However, the chemical nature and mode of action of the fungal metabolite is unknown.

**LITERATURE CITED**