Root Cortical Cell Spherical Bodies Associated with an Induced Resistance Reaction in Monoxenic Cultures of *Meloidogyne incognita*

D. Orion, W. P. Wergin, and D. J. Chitwood

Abstract: The root-knot nematode *Meloidogyne incognita* was monoxenically cultured on excised roots of soybean cv. Pickett and tomato cv. Rutgers in agar media containing either 0 to 1,600 μg/ml ammonium nitrate or 0 to 100 μg/ml urea. Observations with scanning and transmission electron microscopy indicated that an elevated concentration of ammonium nitrate or urea inhibited giant cell formation and suppressed nematode development in the infected soybean roots. In the tomato roots, concentrations of ammonium nitrate above 400 μg/ml or urea above 25 μg/ml inhibited giant cell formation and nematode development. Coincident with the nitrogen concentrations that suppressed giant cell formation was the appearance of electron-dense spherical bodies in the cortical parenchyma cells of both the soybean and tomato roots. These bodies, which were 1–4 μm in diameter, appeared to form in the cytoplasm and migrate to the cell vacuole.

Key words: Cortical cell, *Glycine max*, *Lycopersicon esculentum*, *Meloidogyne incognita*, nematode, nitrogen, root gall, root-knot nematode, scanning electron microscopy, spherical body, transmission electron microscopy.

Natural resistance to root-knot nematodes (*Meloidogyne* spp.) is often expressed as the failure of second-stage juveniles (J2) to induce giant cell formation at a feeding site. In a susceptible host, the giant cells develop and provide the nematode with the nourishment needed for its intense metabolic rate. Alternatively, in a resistant host the cells surrounding the head of the nematode remain small and become necrotic because of various enzymatic and other molecular reactions. These necrotic cells do not provide the nourishment and energy needed by the nematode. Consequently, the nematode fails to develop and reproduce and starves to death. The development of the necrotic cells, which occurs in a resistant cultivar, is referred to as the hypersensitive reaction (1,2).

In a previous study, high concentrations of ammonium nitrate incorporated into the media of monoxenic cultures of *M. incognita* on excised tomato roots significantly inhibited giant cell formation and nematode development without affecting root growth (8). Further studies demonstrated that urea, hydroxyurea, and thiourea exerted similar inhibitory effects on giant cell formation and nematode development (3–5,9).

Recently, an attempt was undertaken to culture *M. incognita* on excised roots of a root-knot nematode-susceptible soybean cultivar under the same conditions used for successful growth on tomato roots. However, nematodes failed to develop and reproduce in the soybean cultures. Histological examination revealed that the giant cells were poorly developed (Orion, unpubl.) In the present study, we investigate the structure of *M. incognita*-induced galls in excised roots of soybean and tomato that were cultured on media enriched with either ammonium nitrate or urea.

**MATERIALS AND METHODS**

A population of *Meloidogyne incognita* was maintained on roots of excised tomato (*Lycopersicon esculentum* cv. Rutgers). The roots were cultured on a Skoog, Tsui, and White (STW) medium (7) modified by substituting 0.8% phytagel for agar and by decreasing the concentration of ammonium nitrate to 100 μg/ml.

To study the effects of nitrogen concentration, tomato and soybean (*Glycine max* cv. Pickett) roots were cultured on modified STW medium containing either am-
monium nitrate (0, 25, 100, 400, or 1,600 μg/ml) or urea (0, 25, 50, or 100 μg/ml). Procedures for seed surface sterilization, germination and transfer of root tips, and their inoculation with nematodes were described previously (8). Uninoculated excised roots served as controls. For observation in the scanning electron microscope (SEM), the developing 3-week-old galls and uninoculated root segments were removed from the cultures, chemically fixed in 3% glutaraldehyde in 0.05 M phosphate buffer, dehydrated in an ethanol series, and embedded in polyethyleneglycol (PEG), MW 8,000. The embedded tissue was then trimmed to expose the feeding sites and prepared for SEM observations as previously described (6). For transmission electron microscopy (TEM), fresh gall tissue and control roots were chemically fixed, dehydrated, embedded, and sectioned according to previously described procedures (10).

SEM images were obtained with a Hitachi S-530 instrument operating at 10 kV and recorded onto Polaroid Type 55 P/N film. The thin sections were observed in a Hitachi H-500H TEM operating at 75 kV and recorded on Kodak Electron Image Plates.

**Results**

**SEM observations:** Figures 1 and 2 illustrate normal giant cells that developed on tomato roots cultured on low concentrations (100 μg/ml) of ammonium nitrate. The main body of the nematode lies in the cortical region of the root, whereas the giant cells develop near the endodermis (Fig. 1). The giant cells are easily distinguished by their large sizes (often 0.1 mm in diameter), dense cytoplasm, thick cell walls, and the xylem tissue that proliferates around the feeding site (Fig. 2).

In tomato roots cultured in medium containing 1,600 μg/ml ammonium nitrate, the giant cells rarely exceed 50 μm in diameter and their cytoplasmic contents (Fig. 3) are much less dense than in infected roots cultured on 100 μg/ml ammonium nitrate. Furthermore, the xylem tissue only rarely surrounds the giant cells, which are more commonly bounded by cortical parenchyma cells containing numerous spherical bodies measuring 0.5 to 1.5 μm in diameter (Fig. 4).

In galls from tomato roots cultured in 50 μg/ml urea (Figs. 5,6), the structure of the giant cells and the presence of the spherical bodies is similar to those at 1,600 μg/ml ammonium nitrate. The cytoplasm of the giant cells is sparse, consisting of cytoplasmic threads (Fig. 5), and spherical bodies are abundant within the cortical parenchyma cells (Figs. 5,6).

In contrast to the normal appearance of the galls that developed on tomato roots at 100 μg/ml ammonium nitrate, the giant cells in soybean roots cultured on medium containing this low concentration (as well as the other concentrations) seem degenerated and nearly devoid of contents (arrows, Fig. 7). In addition, the cortical parenchyma cells contain the spherical bodies (Fig. 8) that appeared in the tomato roots at 1,600 μg/ml. However, these bodies, which may attain 5 μm in diameter, are generally larger than those found in tomato roots and have a rough surface (Fig. 9). Cross sections of the bodies indicate that they are dense and somewhat homogenous (Fig. 10). In most cases, the large spherical bodies do not appear bounded by membranes but are located on the surface of the tonoplast inside the vacuole (Figs. 9,10).

**TEM observations:** In thin sections observed with TEM, the spherical bodies appear in the cortical parenchyma cells from soybean galls as circular, electron-opaque structures (Fig. 11). In general, these bodies are uniformly electron opaque, have loosely granular or particulate material on their surfaces, and measure 2–7 μm in diameter. The spherical bodies tend to lie in the vacuole and be detached from the tonoplast. Smaller bodies with similar electron opacity frequently lie along the tonoplast, however, and have a somewhat flattened or hemispherical shape (Fig. 12).
FIGS. 1–4. Scanning electron micrographs of *Meloidogyne incognita* galls grown on tomato roots from cultures containing 100 μg/ml (Figs. 1, 2) or 1,600 μg/ml (Figs. 3, 4) ammonium nitrate. 1) A cross section through gall. Note the depression formed by the nematode and the normal giant cells; bar = 20 μm. 2) Higher magnification of a normal giant cell illustrating the dense cytoplasm, thick cell wall, and deformed xylem cells that surround the giant cell; bar = 10 μm. 3) Abnormal giant cells with smaller and less dense cytoplasm than those illustrated above. Small spherical bodies can be seen throughout the cortical parenchyma cells; bar = 20 μm. 4) Higher magnification of the parenchymal tissue illustrating the spherical bodies; bar = 1.0 μm.
Figs. 5–8. Scanning electron micrographs of *Meloidogyne incognita* galls grown on tomato or soybean roots and containing abnormal giant cells. 5) A cross section of a tomato gall cultured on 50 µg/ml urea; the giant cells are nearly devoid of cytoplasm, and spherical bodies are abundant in the parenchyma tissue; bar = 20 µm. 6) Portion of a cortical parenchyma cell from a tomato gall cultured on 50 µg/ml urea, illustrating the smooth-surfaced spherical bodies; bar = 2.0 µm. 7) Cross section of a soybean gall cultured on 100 µg/ml ammonium nitrate; note the poorly developed giant cells; bar = 20 µm. 8) Spherical bodies in parenchyma cells from a soybean gall cultured on 100 µg/ml ammonium; bar = 10 µm.
Figs. 9–11. Scanning (Figs. 9,10) and transmission (Fig. 11) electron micrographs of *Meloidogyne incognita* galls grown on soybean roots in media having 100 μg/ml ammonium nitrate. 9) Large spherical bodies with rough surfaces; bar = 1.0 μm. 10) Spherical body sectioned to reveal the dense matrix; bar = 0.5 μm. 11) Cortical parenchyma cell containing electron-opaque spherical bodies that generally lie in the cytoplasm adjacent to the tonoplast; bar = 4.0 μm.

The flattened surfaces of these bodies appear to be within or appressed to the parietal layer of cytoplasm that characterizes these parenchyma cells (Fig. 13). The largest spherical bodies are always within the vacuole and occasionally contain clear or
FIGS. 12–14. Transmission electron micrographs of spherical bodies in cortical parenchyma cells of a *Meloidogyne incognita*-infected soybean gall. 12) Spherical bodies that appear to be emerging from the cytoplasm; bar = 2.0 μm. 13) Spherical body apparently entering the vacuole; bar = 1.0 μm. 14) Large spherical body, believed to be "mature," illustrating the particulate material on the surface and several small clear circular areas within the body; bar = 1.0 μm.

In cells of tomato galls from cultures containing high concentrations of ammonium nitrate (1,600 μg/ml), TEM observations revealed that the spherical bodies, al-
though somewhat smaller than those in soybean cells, have a general appearance and distribution similar to those described for soybean. Similarly, parenchyma cells from tomato galls from cultures incorporating 50 μg/ml urea contained spherical bodies that resembled those associated with high concentrations of ammonium nitrate.

No spherical bodies were detected in uninoculated roots grown in the various media. The various concentrations of ammonium nitrate did not affect the growth of the excised tomato or soybean roots in the uninoculated controls. In contrast, 100 μg/ml urea caused significant inhibition of growth in both the soybean and the tomato roots.

**DISCUSSION**

This study describes spherical bodies whose appearance is correlated with the occurrence of poorly developed giant cells in monoxenic cultures of *M. incognita* on excised soybean and tomato roots. In excised roots of the root-knot nematode-susceptible soybean cultivar, giant cells failed to develop properly in all treatments, which consisted of different concentrations of the nitrogenous compounds ammonium nitrate and urea. Perhaps the composition of the STW medium caused some disturbance in normal root-knot nematode-soybean relationships or induced resistance in the excised soybean roots.

In the *Meloidogyne*-infected tomato roots, the giant cells were well developed in media with ammonium nitrate concentrations less than 400 μg/ml. At higher concentrations the giant cells were poorly developed. In media containing 25 μg/ml urea, giant cell formation was also poorly developed, as previously reported (4). Although the spherical bodies were occasionally found in treatments consisting of low ammonium nitrate concentrations, the bodies were larger and much more numerous in the infected roots exposed to the high concentrations of ammonium nitrate or urea. The appearance of the spherical bodies coincided with poor development of giant cells in root-knot nematode-infected roots. At this time, we cannot conclude that the spherical bodies caused or resulted from an induced resistance reaction.

The spherical bodies appear to form in the cytoplasm adjacent to the tonoplast in the cortical parenchyma cells. As the bodies increase in size, they appear to migrate through the tonoplast, possibly by a pinocytotic mechanism. Once they have completed this process, they appear perfectly spherical and are randomly scattered throughout the vacuole.

The spherical bodies in the cortical cells of tomato and soybean roots appear to differ in two respects: those in the tomato cells were smaller and have relatively smooth surfaces, whereas those in soybean were larger and had rough surfaces. In TEM observations the spherical bodies in both species were osmiophilic, indicating that their contents may partially consist of unsaturated lipids.

In this study, the formation of poorly developed giant cells was correlated with the formation of spherical bodies. Perhaps further studies will extend information about this association to other *Meloidogyne* species and other hosts, both susceptible and resistant to the root-knot nematode, in an attempt to identify the molecular processes that lead to the induced resistance reaction.

**LITERATURE CITED**


4. Glazer, I., and D. Orion. 1984. Influence of urea, hydroxyurea, and thiourea on *Meloidogyne jav-


