CELLULAR CHANGES INDUCED BY XIPHINEMA VULGARE IN THE ROOTS OF CITRUMELO AND BY XIPHINEMA INTERMEDIUM IN THE ROOTS OF BERMUDA GRASS

by


Summary. Feeding by Xiphinema vulgare on Swingle citrumelo and Xiphinema intermedium on Bermuda grass induced pronounced disturbances of attacked roots. Both nematodes removed cell contents through perforation and lysis of consecutive cell walls and formed syncytia-structures. The differentiation zone in citrumelo and the meristematic region in Bermuda grass reacted hypersensitively to nematode injury. Ultrastructural evidence revealed that the membranes of subcellular organelles, and the plasma membrane in particular, were the primary targets of elicitor molecules in cells inside and outside the feeding site. The nuclear membrane was the most resistant to damage, except in affected meristems of Bermuda grass. X. intermedium feeding on parenchyma cells of the differentiating region in Bermuda grass caused hypertrophy of nuclei which became highly amoeboid. These modifications are very similar to those induced by other Xiphinema species in their hosts. Multinucleate cells were not induced because of the lytic process involving all the root cells.

During a research visit of F. Lamberti to the Citrus and Education Center, Lake Alfred, Florida U.S.A., L. W. Duncan drew his attention to a declining citrus plantation near Polk City. The orange grove, which was planted on Swingle citrumelo rootstock, contained patches of stunted plants (Fig. 1), and in general had an unthrifty appearance (Fig. 2).

Also, J. R. Rich sent soil samples collected from a Bermuda grass field at Quincy, Florida, which had symptoms of patchy growth suggestive of a nematode problem.

In both cases the root tips of the respective plants showed accentuated swelling and distortions typical of the symptoms of attack by longidorid nematodes. Additionally, large populations of Xiphinema (ca. 5000/liter soil at Polk City, in December 1995) were detected in the rhizospheres of affected trees and grass.

Species of Xiphinema are ectoparasites that attack economically important crops ranging from herbaceous plants to trees. Cellular responses within the roots induced by nematode feeding appear to differ between species (Bleve-Zacheo et al., 1987).

The present study was undertaken to determine the cytological responses induced by X. vulgare in Swingle citrumelo and by X. intermedium in Bermuda grass.

Materials and methods

Populations of Xiphinema were extracted from 1 litre soil aliquots by means of Cobb's
wet technique using a 710 μm mesh diameter sieve to separate the debris and a 90 μm mesh diameter sieve to collect nematodes. Nematodes were killed and fixed in 4% hot formalin and adult females of Xiphinema mounted in anhydrous glycerol. Measurements were taken with the aid of a camera lucida to identify the species present at each location.

Seeds of Swingle citrumelo [Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf.] and Bermuda grass [Cynodon dactylon (L.) Pers.] were surface-sterilised by immersion for 2 min in 70% ethanol, 4 min in 5% calcium hypochlorite, followed by rinsing three times in sterile water. The seeds were then transferred to 9 cm diameter plastic Petri dishes containing 7 ml nutrient medium (0.1% Gamborg's B5 vitamin solution, 1% agar and 2% sucrose). The seedlings were maintained at 25 °C and in a 16 h light regime throughout the experiment. Specimens (females and juveniles of X. vulgare Tarjan obtained from the citrumelo plantation at Polk City and X. intermedium Lamberti et Bleve-Zacheo from the Bermuda grass field at Quincy were sterilised for 30 min in 0.03% NaN₃ solution and washed three times in sterile distilled water. Batches containing 20 of either X. vulgare or X. intermedium were transferred to Swingle citrumelo or Bermuda grass seedlings, respectively, in an aqueous suspension and their behaviour observed under a light microscope.

For electron microscopy studies, swollen root tips of Swingle citrumelo, three days after nematode inoculation, and attacked root tips of Ber-

Fig. 1 - A citrus plantation at Polk City, Florida, with patches of stunted plants. Soil is heavily infested by Xiphinema vulgare.
muda grass, at 24 h, 48 h, 3 and 5 days after nematode inoculation, were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.05M cacodylate buffer (pH 7.2) for 2 h, rinsed 4 times in the same buffer and post-fixed in a solution of 2% osmium tetroxide in cacodylate buffer for 2 h at 4 °C. Specimens were dehydrated through an ethanol series, substituted by propylene oxide and then infiltrated with Spurr's resin. They were then transferred into flat moulds and the resin was polymerised at 60 °C for 24 h. Ultrathin sections were made with a Reichert (Leica) Ultracut E, mounted on formvar-coated 100 mesh grids and stained with a saturated ethanolic solution of uranyl acetate followed by lead citrate. Specimens were examined in a Philips 400 T transmission electron microscope.

Results

The Xiphinema population from the citrus plantation at Polk City was morphometrically identical to type specimens of X. vulgare as described by Lamberti et al. (1995) and that from Bermuda grass at Quincy fit perfectly the original description of X. intermedium (Lamberti and Bleve-Zacheo, 1979).

Feeding of X. vulgare caused a severe reduction of the root system in Swingle citrumelo. Cytological changes associated with this occurred mainly at the level of differentiating tissues and was associated with necrosis and discoloration of the remaining portion of the root tips. As a consequence, roots parasitized by the nematode were clearly recognisable because of
their slight swelling with large spots of necrotic cortical cells.

Sections through the stelar tissue of an intact root showed that vascular parenchyma cells around the developing vessels were extensively vacuolated (Fig. 3a). In addition, nuclei occupied most of the dense cytoplasm and mitochondria had numerous cristae in their matrices (Fig. 3b). A moderate amount of endoplasmic reticulum was observed in most cells, whereas dictyosomes were generally few in number. Peroxisomes with a crystalline core of catalase frequently were present (Fig. 3c).

Parenchyma cells, selected as feeding sites by the nematode, became hypertrophied. Widespread cell wall disruption indicated that they had been breached by odontostyle penetration (Fig. 3d). Three days after nematode inoculation, the feeding site consisted of cells completely devoid of cytoplasm or still partially filled with degraded contents. The presence of lipid bodies in these cells indicated cell membrane reactions that occurred before the disruptive action of cell sap removal by the nematode (Fig. 3d). The same metabolic process of lipid-like accumulation involved the cells around the feeding site of the nematode, while mitochondria and peroxisomes did not show such drastic disturbances (Figs 3e, g). Paramural bodies were commonly present in the cells not directly injured by the nematode. These structures appeared to be invaginations of the plasma membrane containing small vesicles (Fig. 3f) and membranes (Fig. 3h). They were not associated with any localised modification of the adjacent cell wall. The plastids, which appeared to be anchored by cytoskeletal elements near cell walls, were distorted and elongate, and contained large deposits of ferritin and starch granules in their stroma (Fig. 3h).

The feeding behaviour of X. intermedium on Bermuda grass resulted in cellular responses that were quite different from those induced by X. vulgare on Swingle citrumelo. In Bermuda grass roots not exposed to X. intermedium the cells of the cortical ground meristem had a large nucleus separated by a double membrane from a densely particulate cytoplasm. Each nucleus contained a large vacuolated nucleolus and chromatin in the nucleoplasm, either in dispersed form or as dense heterochromatin (Fig. 4a). The plasma membrane passed through the intervening cell wall at plasmodesmata of adjacent cells. The cytoplasm was dense with numerous ribosomes, mitochondria, dictyosomes, and long ER profiles (Fig. 4b).

The feeding activity of X. intermedium induced lysis of the meristematic cells that formed a feeding site (Fig. 4c). Breakdown of the cell walls resulted in the fusion of the cytoplasm and the formation of a multinucleate syncytium 24 h after nematode inoculation. All membranes, including plasma and nuclear membranes, were electron dense because of osmiophilic depositions (Fig. 4d). Nuclei, either those of cells of the feeding site or neighbouring cells, appeared not to be severely affected apart from disconnected outer nuclear membrane (Figs 4d, e). Many actively synthesizing dictyosomes and a large amount of transient vesicles moving toward the plasma membrane were present in cells outward the feeding site; they appeared to be involved in the formation of secondary wall apposition. The plasma membrane became invaginated and was without electron-dense osmiophilic deposits, but these were detectable between the cell wall and the plasma membrane (Fig. 4f). Enlarged polysome-decorated ER profiles and mitochondria with markedly dark stained membranes indicated an unusual metabolism in these cells (Fig. 4f).

When X. intermedium utilized the cells in the differentiating region as a food source, cell wall breakdown in a row of parenchyma cells was the first response. Cells just pierced by the odontostyle of the nematode (24 h after nematode inoculation) were vacuolated but were still well organised (Fig. 5a). Cytoplasmic contents were greatly reduced, and vacuoles appeared to have fused in response to nematode feeding, 48
Fig. 3 - Micrographs of cross sections of Swingle citrumelo root tips unattacked or fed upon by X. vulgare: a) differentiating zone of unattacked root tip of citrus where parenchyma cells show dense cytoplasm and a large nucleus occupying the greater part of each cell x 2,800; b) enlargement of a parenchyma cell containing a portion of nucleus with nuclear pores (arrow) in its membranes, the outer one decorated by ribosomes, and dense mitochondria (double arrow) x 22,000; c) section through three parenchymatous cells. Peroxisome (arrow) with catalase core lining the plasma membrane, profiles of ER (double arrow), and DNA strand in a mitochondrion (head arrow) can be seen x 36,000; d) wall breakdown (arrow) between parenchyma cells (fs) indicates the action of the nematode odontostyle. Cytoplasmic contents of the cells have been completely removed, three days after nematode infection x 4,400; e) cell bordering the feeding site containing mitochondria and a peroxisome, but no other cellular organelles are recognizable x 31,000; f) paramural body (arrow) and deranged membranes associated with lipids (double arrow) in a cell adjacent to a necrotic feeding site are indicative of cell reaction x 22,000; g) lipid bodies (double arrow) in two cells, where darkening of membranes (arrow) is detectable, indicating an unusual metabolism although the nucleus is still well preserved x 15,800; h) elongated and distorted plastid showing dense matrix, ferritin deposits (arrow) and starch granules (arrow head); in an adjacent cell apposed membranes (double arrow) extend the plasma membrane x 31,000.
Fig. 4 - Micrographs of cross sections of Bermuda grass root tips fed upon by *X. intermedium*: a) meristematic cells in unattacked root tip showing dense cytoplasm with small vacuoles and nuclei with large nucleoli, exhibiting nucleolar organizers (arrow), x 8,000; b) wall between two meristematic cells with numerous plasmodesmata (arrow head) present. Note polysome-decorated ER profiles, mitochondria (double arrow) and dictyosomes (arrow) x 22,000; c) section through the feeding site (fs) in meristematic tissue of a root tip, 24 h after *X. intermedium* infection; some cells are fused because of cell wall breakdown and have cytoplasm partially destroyed x 4,600; d) enlargement of the feeding site, showing a syncytium-like structure caused by rupturing of the intervening cell wall (arrow); the cytoplasm is disconnected from the wall and membrane dark-lined (arrow head) x 9,000; e) meristematic cells adjacent to the feeding site with severely reacting nuclear membranes (arrow) x 8,700; f) high magnification of the cell in Fig. 4e showing numerous dictyosome-produced vesicles (arrow) in relation to electron-light deposit outside the plasma membrane (double arrow); note osmiophilic deposition, different in shape, scattered along the plasma membrane and the cytoplasmic organelles (arrow head) x 28,000.
h after inoculation. The presence of necrotic cells adjacent to those forming the feeding site suggests that there may be two ways to act cell puncturing, the first a simple mechanical process to gain access to the parenchyma cells and the second a more complex process that utilized enzymes for cell wall digestion (Fig. 5b). Three to five days after nematode inoculation, cells at the feeding site that previously had been active were now dead. However, their original walls were intact but breacked in the middle between two parallel cells, the holes sufficiently large for odontostyle insertion (Figs 5c, d). Cells, partially or completely depleted of their contents, were surrounded by cells undergoing modification. These cells were larger than corresponding cells in normal roots and exhibited a digitate plasma membrane as a result of deposition of electron-transparent callose-like material. Their nuclei were enlarged and considerably lobed with peripheral clumps of condensed chromatin. The ground structure of the cytoplasm was very dense with numerous mitochondria, polysomes and ER profiles, and few vacuoles (Figs 5e, f). A large proportion of the densely staining plastids had well developed ring-shaped internal membranes (Figs 5f, g). These morphological changes, together with the number of mitochondria present, are indicative of an increase in respiration and active protein metabolism. One of the products of metabolism appears to be lipid bodies scattered in the cytoplasm (Figs 5e, g).

**Discussion**

The results of this study provide direct evidence that *X. vulgare* and *X. intermedium* feed and cause damage to the roots of Swingle citrumelo and Bermuda grass, respectively. However, the cytological alterations induced by these two species differ in many respects from those reported for other *Xiphinema* spp. (Wyss, 1987; Bleve-Zacheo et al., 1987). It has been shown that *X.

index reorganises the root morphology and physiology in its host and establishes complex feeding structures (multinucleate cells) that provide an efficient source of nutrients. These cellular adaptations resemble in many ways those associated with sedentary root-knot nematodes, apart from their shorter life because of their progressive but prolonged degradation (Wyss et al., 1988). In instances where extensive lysis of the procambial cells in the host root has been reported, it has been difficult to ascertain whether this is a direct result of nematode feeding or a response to secondary components generated during infection (Zacheo and Bleve-Zacheo, 1995).

The inability of *X. vulgare* to induce a coenocyte in Swingle citrumelo roots leads to the conclusion that it is a non-host for this species and that it should be included in the "feeder plant range" (Fritzche and Hofferek, 1969). The reaction of the cells to the presence of the nematode, as evident in alterations in vesicular and membranous structures associated with the plasma membrane, indicates an active response of host cells to signals from an incompatible parasite and that the plant does not provide sufficient nutrients to sustain the pathogen. The presence of paramural bodies in root cells of cereal and leguminous hosts attacked by *Longidorus belloii* not associated with modified regions of the cell walls, as in citrumelo cells, has been considered to manifest a generalised alteration of the plasma membrane (Andres et al., 1989).

It has long been known that the hypersensitive reaction (HR) reflects a disturbance of the vital membranes of host cells. Electron opaque, lipid-like accretions that were present in many membranes are signs not only of membrane disarrangement, but also dissolution of the lipid bilayer. The development of a hypersensitive reaction enclosing nurse cells may be incited either directly by a component in the nematode's saliva or by a salivary component that initiates a series of biochemical reactions. The second hypothesis seems to be more likely because of the difference between necrotic cells mechanically
Fig. 5 - Micrographs of cross sections of Bermuda grass root tips fed upon by X. intermedium. a) feeding site in differentiating cells 24 h after nematode infection. A row of cells forming the feeding site (fs) with intervening broken walls (arrow) is visible; the cytoplasm in these cells is highly vacuolated (v); a cell (nc) mechanically damaged by odontostyle thrust lies between vessels and feeding site x 4,000; b) syncytia-like cells (fs), 48 h after nematode infection, adjacent to necrotic cells (nc). The cytoplasmic content is very reduced, remaining nuclei still intact x 3,400; c) degraded cytoplasm remaining in a syncytium-like structure, 3 days after nematode infection x 7,600; d) three cells, five days after nematode infection, showing holes in their walls similar to diameter of odontostyle aperture. Almost empty cells are adjacent to necrotic cells (arrow) x 4,500; e) cell adjacent to the feeding site, 48 h after nematode infection. The amoeboid nucleus has two separated nuclear portions (arrow); numerous mitochondria (arrow head) are indicative of high respiratory activity x 12,000; f) cell outside the feeding site, showing irregular nucleus with clumped chromatin, elongated mitochondria (arrow) and digitate plasma membrane (arrow head) x 9,300; g) digitate plasma membrane with electron-light deposit (arrow), ring-shaped and elongated plastids rich in intermembranes (double arrow), ER profiles (arrow head) with polysomes, all indicating an unusual metabolism, manifested by the presence of lipid bodies (l) x 15,500.
damaged by the nematode and physiological cell death. Once the signal has been received throughout the injured cells, healthy cells initiate a sequence of biochemical events that lead to membrane deterioration and to the synthesis of products such as phytoalexins that are toxic to both plant cells and nematodes (Zacheo and Bleve-Zacheo, 1995).

In Bermuda grass, the pattern of response was different and depended on the cells selected as feeding sites by X. intermedium. The magnitude of the reaction in affected meristematic tissue appears to be proportional to the intensity of metabolic activity in the cells. The almost simultaneous development of intense electrolyte leakage and induction of syncytia-like nurse cells lead us to conclude that they are directly and temporally related. There is clearly a loss of cell turgor and an efflux of electrolytes. This is a characteristic of HR-associated membrane perturbation as caused by incompatible pathogens. It reflects membrane damage and the possible target, a lipid component of cell organelar membranes (Goodman and Novaky, 1994). Associated with HR is the induction of increased synthesis of mRNAs coding for enzymes involved in subsequent phenolic synthesis. Meristematic cells have a capacity for comprehensive aromatic synthesis and oxidative activity, that can be elicited by any ecto- or endoparasitic nematode (Bleve-Zacheo et al., 1995).

The overall response of differentiating cells to nematodes feeding is clearly somewhat delayed in relation to that in the meristematic cells. Cell wall breakdown forming a syncytium-like structure is accompanied by stimulatory events in neighbouring cells that strongly influence the nuclei, confirming the metabolic enhancement in roots parasitized by other Xiphinema species (Wyss, 1981). It can be argued that differentiating tissues are prevented from having a hypersensitive reaction because of their hormonal status (Raskin, 1992). However, a gradual catabolic reaction related to the lysis of cell components leads to a similar end result cell death and subsequent root degeneration.

**Literature cited**


