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Ultrastructure and cytochemistry of interactions between banana and the nematode *Radopholus similis*

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Abstract – An ultrastructural and cytochemical investigation was undertaken to study pathogenesis in banana roots attacked by the burrowing nematode *Radopholus similis*. In the susceptible (Poyo) and the partially resistant (Yangambi) cultivars, the pathogen ingress within host tissues was characterized by the occurrence of tunnels, resulting in severe host cell wall alterations. In susceptible plants, host cells displayed modifications of the cytoplasm seen in the cortex and the vascular bundle. Root penetration was less important in the resistant cultivar in which the nematode was found in the cortex only. Bacterial cells were found located close to the nematode during the infection process. The presence of constitutive ß-1,3-glucans in cortical cell walls and of a thick suberized layer in the endodermis cells may explain the low rate of nematodes in roots of the resistant cultivar. Induced reactions to infection consisted in callose deposition, accumulation of phenolics and cell hyperplasia in the vascular stele. However, the production of phenol compounds was detected in roots of the resistant cultivar only.

Résumé – ***Ultrastructure et cytochimie des interactions entre le bananier et le nématode*** **Radopholus similis –** Une étude ultrastructurale et cytochimique de la pathogenèse a été entreprise sur des racines de bananier attaquées par le nématode migrateur *Radopholus similis*. Chez les cultivars sensible (Poyo) et particllement résistant (Yangambi), la progression du parasite dans les tissus hôtes est caractérisée par la présence de tunnels résultant d'altérations importantes des parois cellulaires de l'hôte. Chez les plants sensibles, des cellules du cortex et du cylindre central montrent de sévères modifications cytoplasmiques. La pénétration racinaire est moins importante dans le cultivar résistant chez lequel le nématode a été uniquement observé dans le parenchyme cortical. Des bactéries ont été observées associées aux nématodes durant le processus infectieux. La présence de ß-1,3-glucanes constitutives des parois cellulaires corticales et d'une couche pariétale subérifiée des cellules de l'endoderme pourrait expliquer le faible taux de nématodes dans les racines du cultivar résistant. Les réactions induites par l'infestation consistent en des dépôts de callose, une accumulation de composés phénoliques et une hyperplasie de cellules du cylindre central. La production de composés phénoliques n'a cependant été détectée que dans les racines du cultivar résistant.

Key-words : Callose, cell wall alteration, phenols, resistance, nematodes, *Radopholus*.

Banana (*Musa* spp.) is an important food crop in tropical countries. Worldwide production of fruit (dessert bananas) is 45 millions tons, 12 % of which are assigned to exportation (Stover & Simmonds, 1987). Among pests and diseases that significantly reduce yield, nematodes are considered as one of the main soil-borne factors inducing root damages. In banana plantations, the burrowing nematode *Radopholus similis* is a widespread and damaging pathogen (Adiko, 1988; Sarah, 1989; Gowen & Quénehervé, 1990; Bridge *et al.*, 1995). Roots attacked by this parasite are characterized by a typical reddish brown cortical lesion (Blake, 1961), responsible for uprooting, plant growth reduction and loss of bunch weight.

Control of *R. similis* is currently based on the use of nematicides (Schipke & Ramsey, 1994; Guzman-Gonzalez *et al.*, 1994). Because of the high cost and the negative impact on soil ecology, it was necessary to develop environmentally safe methods of extensive control, including the use of new banana cvs displaying a high level of resistance (Sarah, 1989). Important variation in levels of resistance to *R. similis* has been reported within a wide range of banana cvs (Gowen, 1976; Fogain, pers. comm.). In this respect, field and greenhouse trials revealed that the cultivar Yangambi (Ibota, genotype : AAA) was much less infected by *R. similis* than the cv. Poyo (Cavendish, genotype : AAA) : after plant inoculation, the population of *R. similis* in roots was twice greater on average in Poyo than in the partially resistant cv. Yangambi (Sarah *et al.*, 1992; Fallas & Marban-Mendoza, 1994). Previous histological studies revealed that *R. similis* was the primary pathogen of banana root rot. The nematode was seen to be mainly located in the cortical parenchyma, associated with various host cell damages, including cytoplasm retraction and cell wall degradation (Blake, 1966; Wehunt *et al.*, 1978).

Characterization of genes of resistance to nematode is a promising approach to successfully engineer nematode resistance (Reimann-Philipp & Beachy, 1993; De Waele *et al.*, 1993). Therefore, it is of importance to further elucidate the molecular events involved in plant-nematode interactions, including plant defense responses. Recent advances in the understanding of molecular mechanisms of plant reactions to nematode

infection have led to the characterization of several genes, the expression of which is affected by nematode attack, although most encoded proteins are not yet identified (Opperman & Conkling, 1994; Van Der Eycken *et al.*, 1993). Extensin is an hydroxyproline-rich glycoprotein involved in the reinforcement of host cell walls after plant infection or wounding (Showalter & Rumeau, 1990). Recently, induction patterns of the extensin gene were demonstrated in infected plants (Niebel *et al.*, 1993). These studies mainly focused on responses of plants infested by sedentary nematodes. Plant defense mechanisms induced by burrowing nematodes are still poorly documented, although field resistance has been well established (Pinochet & Rowe, 1978). The objective of the present work was to study features of colonization by *R. similis* and identify plant defense mechanisms against this parasite. It was conducted under controlled conditions on plantlets artificially infected with *R. similis*. Ultrastructural, cytochemical and immunocytochemical investigations were undertaken to gain a better insight into *Musa* sp.-*R. similis* interactions with emphasis on the nematode ingress within root tissues and on host cells responses to infection.

Materials and methods

Nematodes

A highly virulent strain of *R. similis* from a banana plantation in Ivory Coast was used in this study (Sarah *et al.*, 1993). It was maintained monoxenically at 27 °C on sterilized carrot discs in a flask (O'Bannon & Taylor, 1968). Four weeks after carrot disc inoculation, a mixture of adult and juvenile nematodes was recovered by rinsing with distilled water the sides of the flask, where nematodes tend to gather. The concentration of nematodes in the suspension was adjusted to 1200/ml for inoculation.

Plant material

Two banana cvs were used in our experiment: the susceptible cv. Poyo and the partially resistant cv. Yangambi. Banana plants were produced by *in vitro* micropropagation. The vitro-plants were transferred into pots containing sterilized soil and maintained for 45 days in a climatic chamber (27 °C, 75 % relative humidity, 12 h photoperiod), prior to nematode inoculation and cytological studies. The histology of non-inoculated banana roots was studied in 2, 4, 6, and 8 week-old plants cultivated in pots containing sterilized soil.

Plant inoculation

Partially resistant and susceptible plantlets were uprooted, washed with tap water and placed into Petri dishes in which sterilized sand had been previously added (Fig. 1). The nematode inoculum was directly applied on the roots, 1 cm away from the tips. Three roots per plant were inoculated, each with an inoculum of 400 nematodes in 0.33 ml water. To prevent withering, infested and control roots were first covered with humid filter paper and then with aluminium foil. The plants were kept in a climatic chamber. For microscopic observations, roots were sampled 24, 48, 72 and 96 h after inoculation.

Preparation of roots for microscopic observations

For electron microscopic observations, infected and healthy roots were cut into fragments (2 mm in length) in a Petri dish containing glutaraldehyde (2.5 %; v/v) in 0.1 M cacodylate buffer pH 7.2. The pieces were then transferred for 2 h into Eppendorf tubes containing 2.5 % buffered glutaraldehyde. After several washes with cacodylate buffer, the specimens were postfixed for 1 h in 1 % osmium tetroxide (w/v), followed by washes with distilled water. The fragments were then dehydrated in an ethanol series (10 %, 30 %, 50 %, 70 %, 80 %, 90 %, 100 %, 100 %, 1 h each), followed by propylene oxide (1 h) and embedded in Epon according to the supplier recommendations (TAAB, UK). Ultrathin sections were stained with uranyl acetate and lead citrate and then examined with a Jeol electron microscope (LPRC-CIRAD, Montpellier, France).

For light microscope observations, 1.5 μm thin sections of Epon-embedded root fragments were used for counting phenolic cells distributed in non-inoculated roots on 2, 4, 6, and 8 week-old plantlets, cultivated as described above. Sections were stained with 0.5 % toluidine blue (v/v) in sodium carbonate 2.5 % (pH 9). Phenol-containing cells appeared blue-black after staining. Observations were carried out using a light microscope (Diaplan, Leitz).

Nematodes were counted in hand-made sections of roots 24, 48, and 72 h after inoculation. The sections

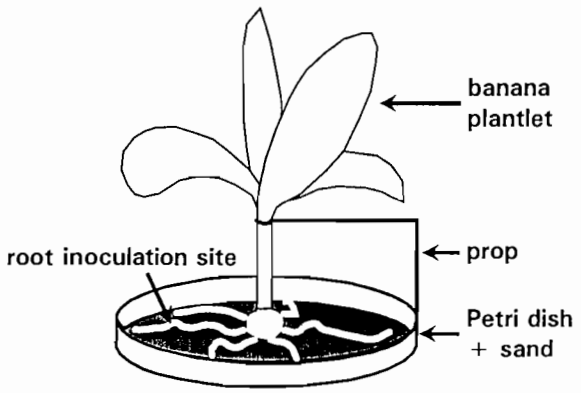

Fig. 1. *Experimental system used, with banana plantlets placed in Petri dishes containing sterilized sand and maintained by a prop. (Inoculum directly applied on the root, 1 cm away from the tip; to avoid withering, roots were covered with humid filter paper and aluminium foil.)*

were stained for 30 s in boiled fuchsin paraldehyde and then washed with distilled water. Nematodes and eggs were stained dark pink in the host tissues.

IMMUNOCYTOCHEMISTRY

JIM 5 monoclonal antibody raised in rat against epitopes of pectin was used on ultrathin sections for pectin localization (Knox *et al.*, 1990). Briefly, sections were first floated on a drop of PBS (Phosphate Buffer Saline)-BSA (1 %)-Tween (0.05 %), then incubated for 2 h at 37 °C on a drop containing the primary antibody. After washing in PBS, sections were floated for 30 min at 37 °C on a drop of gold-labelled goat anti-rat antibodies (GAR-10nm, Biocell, UK) diluted 1:20 in Tris/HCl-BSA (1 %)-Tween 20 (0.05 %).

Polyclonal antibodies raised in rabbit against epitopes of ß-1,3 glucans were used on ultrathin sections for callose localization (Gollotte *et al.*, 1993). Briefly, sections were first floated for 10 min on a drop of glycine 0.1 M in PBS 0.01 M, pH 7.2, then 10 min on a drop of the goat preimmune serum (1/20) in PBS 0.01 M pH 7.4, followed by 15 min on PBS-BSA (2.5 %) Tween (0.05 %) pH 7.4. Sections were then incubated in a moist chamber for 30 min at 37 °C on primary antibodies (1/2000) in PBS-BSA (0.5 %) Tween (0.05 %) pH 7.4. After washing in PBS Tween, sections were floated for 30 min at 37 °C on gold-labelled goat anti-rabbit (1/20) in PBS-BSA-(0,5 %)-Tween (0.05 %) pH 7.4, then washed for 30 min in PBS Tween and distilled water. Controls used for immunolabelling of healthy and infected roots were performed as follows : *i)* incubation with antibodies previously adsorbed with the corresponding antigen, or *ii)* incubation with the rat, rabbit or mouse preimmune serum instead of the primary antiserum.

CYTOLOCALIZATION OF ß-1,4-GLUCANS

An ß-1,4-exoglucanase conjugated to colloidal gold (15 nm) was used on ultrathin sections to visualize ß-1,4-glucans in plant cell walls (Nicole & Benhamou, 1991). Sections were first floated on a drop of PBS-PEG 20000, pH 6, then incubated for 30 min at room temperature in a moist chamber on a drop of the diluted enzyme-gold complex (1/15). They were then thoroughly washed with PBS, pH 7.2, rinsed with distilled water and finally contrasted with uranyl acetate and lead citrate before examination.

Results

ROOT COLONIZATION BY *R. SIMILIS*

Counting of nematodes under light microscope (Table 1) in roots of banana plants revealed that nematodes were found in the susceptible cv. 24 h after inoculation, while none could be detected in the partially resistant cv. The number of nematodes (Fig. 2 A) and eggs recovered in the roots of the susceptible cv. was about four times higher on average than in the roots of the partially resistant cv., 48 and 72 h after inoculation. Only a few eggs could be detected after 72 h in the partially resistant cv. In our experimental conditions, nematodes were found in cortical parenchyma cells both in susceptible (Fig. 2 A, B) and in partially resistant cvs. Nematodes were observed in the vascular stele of the susceptible cv. (Fig. 2 A, B) while none was found in the resistant cv. Ultrastructural investigations revealed that bacterial cells were found located close to the nematode (Fig. 2 C). They were abundant in the susceptible cv. while only few were seen in the resistant cv. These microorganisms were never observed in non inoculated plants (controls) and in root portions that were not colonized by the nematode. Electron-microscope observations also showed the occurrence of egg-like structures in roots of the susceptible cv. (Fig. 2 D). The small diameter of this structure may be explained by a section at the pole, the largest section of *R. similis* eggs measuring around 22 µm.

In the presence of the nematode, host cell damage was observed in both cvs. In the susceptible cv., the cytoplasm of host cells close to the nematode displayed alterations such as withdrawal of the plasma membrane from the cell wall (Fig. 3, A, B), cytoplasm degradation, and cell vacuolation (Fig. 3 B). Cell vacuolation was also observed in the infected partially resistant cv. (Fig. 4 A). In contrast, no cell modification was detected in healthy roots (Fig. 3 C). At later stages of colonization, numerous host cells collapsed but in resistant roots, only a few cells showed similar disorganization (Fig. 4 B).

Nematode ingress within the susceptible host tissues was charaterized by the occurrence of large tunnels resulting in cell walls (Fig. 4 C) and middle lamella breakdown (Fig. 4 D). Similar patterns of degradation were also observed in the root tissues of the resistant cv. but only to a lesser extent.

CYTOCHEMICAL ASPECTS OF PLANT POLYSACCHARIDE ALTERATIONS

The use of an exoglucanase conjugated to colloidal gold allowed to cytolocalize ß-1,4-glucans in host cell walls. In the infected susceptible cv., layers of labelled material were seen to be detached from plant cell walls (Fig. 5 A). Numerous gold particles were detected

Table 1. *Number of nematodes in infected banana roots.*

	Time after inoculation (h)		
Cultivar	24	48	72
Susceptible	18 ± 3.5	22 ± 5	38 ± 9
Resistant	0	5 ± 2	9 ± 2

Data expressed as means from twenty roots.

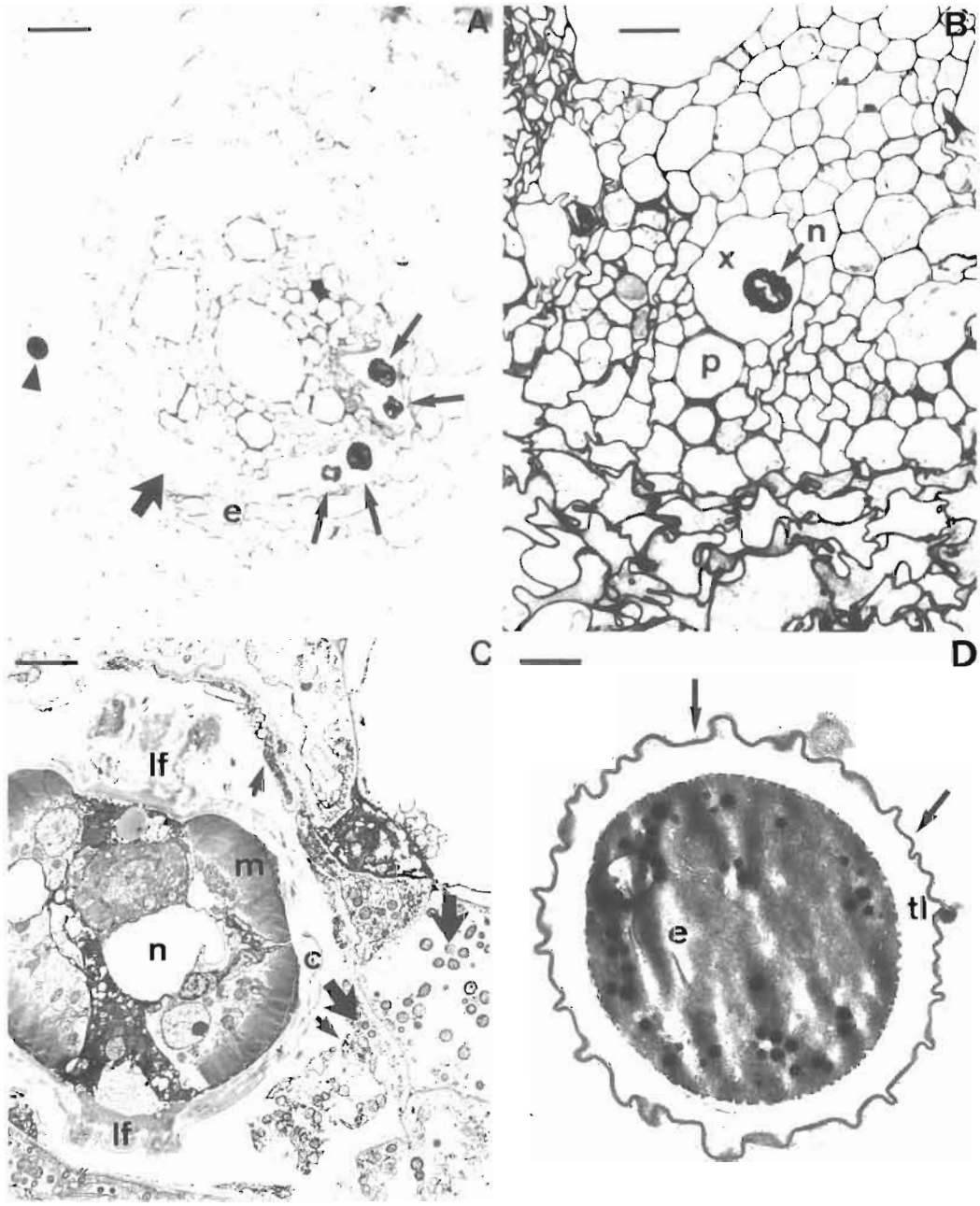

Fig. 2. *Light (A, B) and electron (C, D) microscope micrographs (TEM) of a root of the susceptible cv. infected with* Radopholus similis. *A : 3 days after inoculation, the nematodes are located close (arrowhead) or within the stele (small arrows); host tissue damages caused by the nematode ingress are characterized by the occurrence of a large tunnel (large arrow); (e : endodermis,* bar = 8 μm*); B : 4 days after inoculation, the nematode (n) is located within a metaxylem cell (×); (p : protoxylem,* bar = 2.9 μm*); C : 3 days after inoculation, an adult nematode (n) fills the cell lumen of a parenchyma cell; note the lateral fields (lf), the cuticle (c) and the muscles (m) of the nematode; the host cell displays a degraded cytoplasm and cell wall (small arrows); bacteria are seen near the nematode in the degraded cell (large arrow);* bar = 2.3 μm*); D : 4 days after inoculation, an egg-like structure is located in a cortical parenchyma cell; this structure is delimited by an electron-dense layer (arrows); a median translucide layer (tl) surrounds the cytoplasm (e), rich in electron-dense granules* (bar = 0.7 μm).

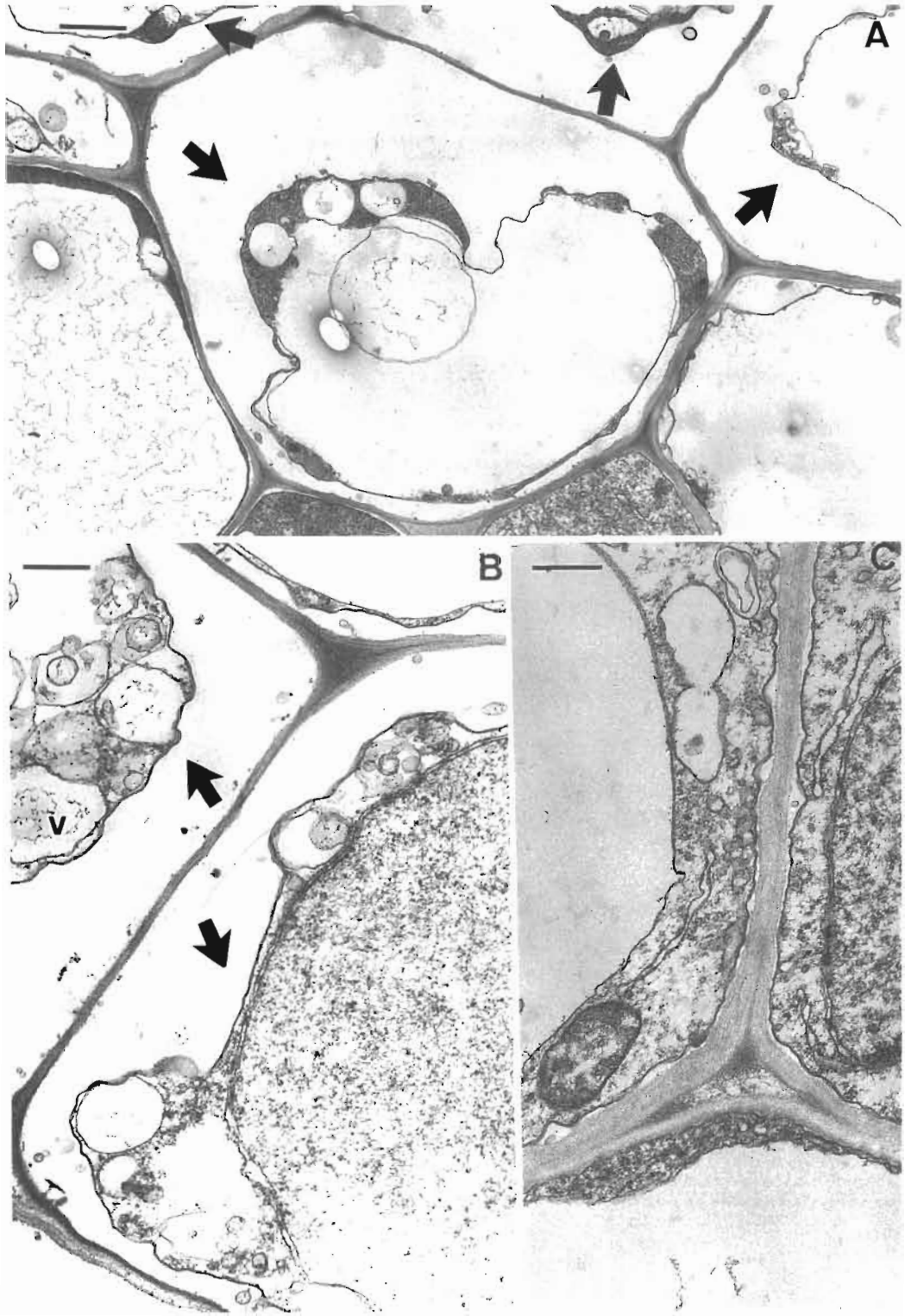

Fig. 3. *A, B : TEM micrographs of infected roots of the susceptible cv., 4 days after inoculation. Host cells located close to the nematode display a retracted cytoplasm (arrows) and a high vacuolation of the cytoplasm (v); C : TEM micrograph of a healthy root of the susceptible cv., the cytoplasm of the cells is well preserved* (Bars : A = 1.05 µm; B = 0.5 µm; C = 0.5 µm).

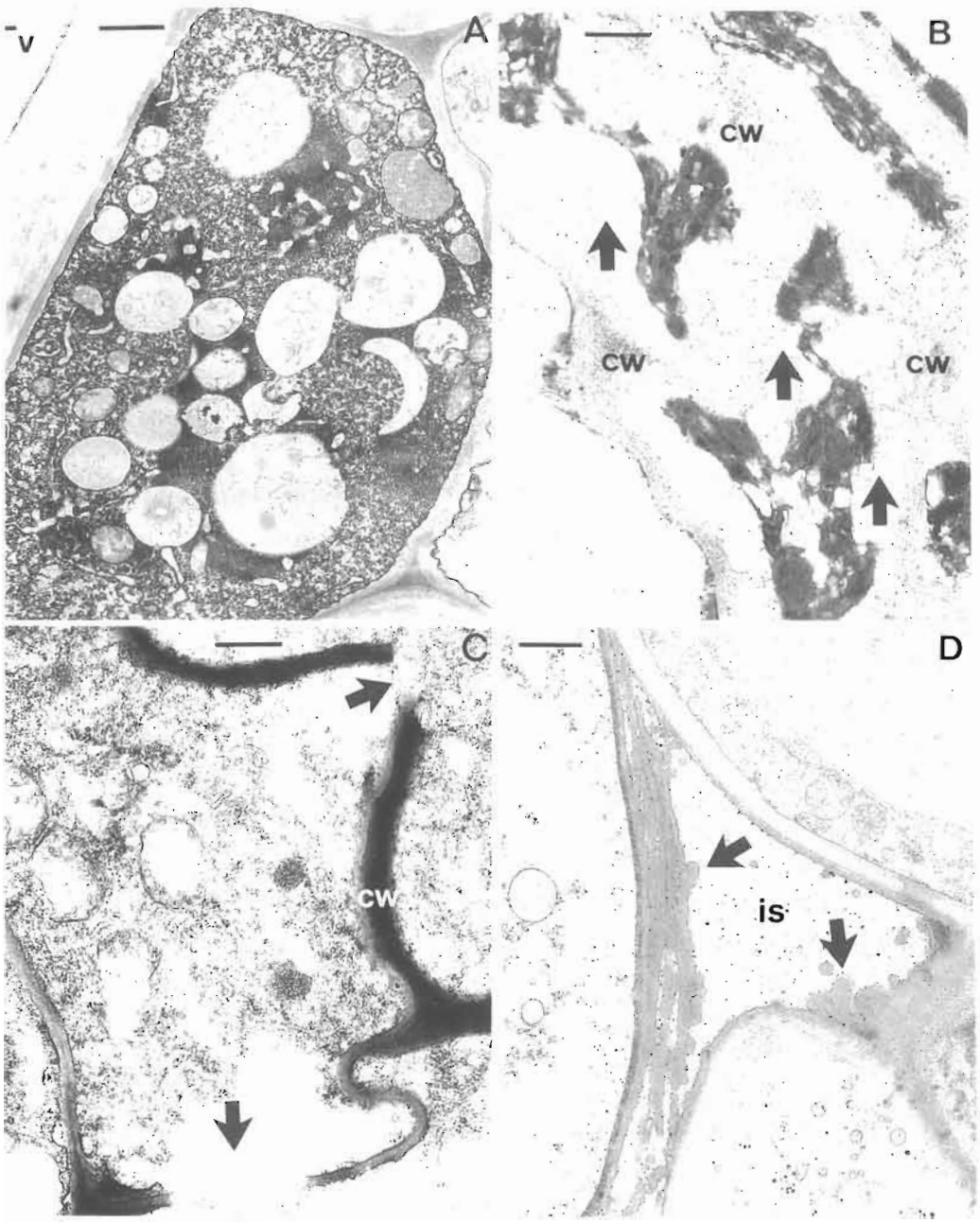

Fig. 4. *TEM micrographs of infected roots of resistant (A, B) and susceptible (C, D) cvs. A : note the vacuolation and the alteration of the cytoplasm of a parenchyma cell adjacent to a vessel (v), 4 days after inoculation* (Bar = 0.9 μm); *B : 2 days after inoculation, the cell close to the nematode shows an electron-dense coagulated cytoplasm and a degraded cell wall (cw) portions of which are associated with the degraded cytoplasm (arrows);* (Bar = 0.55 μm); *C : 4 days after inoculation, the arrows indicate the disruption of a phloem cell wall (cw) that appears also electron-dense;* (Bar = 0.5 μm); *D : 4 days after inoculation, degradation of the middle lamella (arrows) in the intercellular space (is) in the root parenchyma; one of the cells displays a well organized cytoplasm, while the cytoplasm of the two others is degraded* (Bar = 0.5 μm).

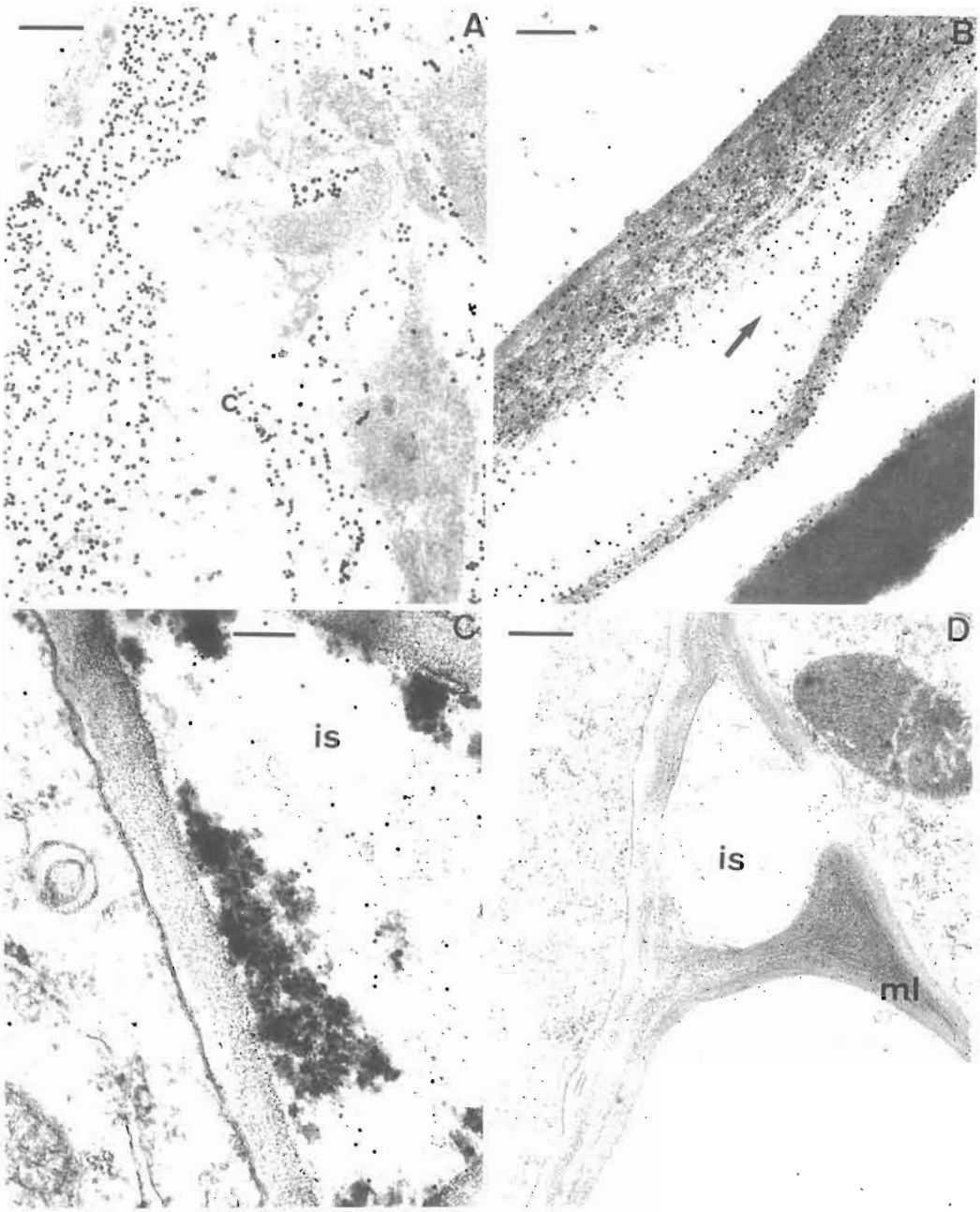

Fig. 5. *Cytolocalization of ß-1,4-glucans by means of a gold-complexed exoglucanase (TEM micrographs). A : Labelled host cell wall fragments are present in the cytoplasm (c) of a degraded phloem parenchyma cell in an infected root of the susceptible cv., 2 days after inoculation* (Bar = 0.17 µm); *B : The labelled cell wall shows a loosened degraded area (arrow) in an infected root of the susceptible cv., 4 days after inoculation* (Bar = 0.26 µm); *C-D : Immunocytolocalization of pectin using JIM 5 monoclonal antibodies (TEM micrographs); C : In an infected root of the susceptible cv., gold particles decorate fibrills located in the intercellular space (is), 4 days after inoculation; in contrast, the dense material in the intercellular space is devoid of any significant labelling* (Bar = 0.14 µm); *D : In an infected root of the partially resistant cv., gold particles are evenly distributed over the middle lamella (ml), 2 days after inoculation; labelling is also seen in the intercellular space (is);* (Bar = 0.36 µm).

over lysed cell walls; labelled cell wall fragments were present in degraded cells (Fig. 5 B).

After the use of monoclonal pectin antibodies, gold particles were seen over middle lamellae and primary cell walls of degraded cells (Fig. 5 C). In degraded areas of the cortical parenchyma of a partially resistant root, few gold particles occurred over intercellular spaces (Fig. 5 D). A fibrillar-like material localized within intercellular spaces was also seen to be decorated by gold particles (Fig. 5 D). In contrast, electron-dense compounds that accumulate in such intercellular spaces were not labelled (Fig. 5 D).

Root responses

Several types of host cell responses were observed associated with root colonization by *R. similis*. Wall modifications mainly consisted in apposition layers that were observed in cells of both susceptible and resistant cvs. Compounds different in their electron-density were seen to accumulate in paramural areas of phloem cells (Fig. 6 A, B). Amorphous material was also detected in paramural areas of degraded phloem cells (Fig. 6 C). The use of probes for ß-1,4-glucan detection (Fig. 6 C) or pectin localization (not shown) did not yield any significant labelling over this amorphous material. However, when anti ß-1,3-glucan antibodies were applied to sections of infected roots, numerous gold particles were detected over this material. Accumulation of ß-1,3-glucans was also observed within papillae located in intercellular spaces (Fig. 6 D). Observations of root sections of healthy resistant plants revealed that a low but even labelling occurred over parenchymal cell walls (Fig. 6 E), but not in the susceptible roots. Layers of dense material which coated the secondary cell wall of xylem vessels (Fig. 7 A), were never labelled with polysaccharide-targeting probes.

The number of phenol-producing cells, counted after staining by toluidine blue, was higher in healthy roots of susceptible cv. than in resistant cv. (Table 2). After inoculation, numerous cells both in the cortex and in the vascular bundle of partially resistant plants showed the presence of electron-dense compounds mainly located in the vacuole and seldom in the cytoplasm (Fig. 7 B, C). The whole cytoplasm of some vessel associated cells was highly electron-dense (Fig. 7 D). These compounds that are dark-blue stained by the toluidine blue are suspected to be of phenolic origin.

Last, hypertrophied cells located within the vascular bundle close to the endodermis were observed in the infected roots of the susceptible cv. (Fig. 7 E, F), but not in the roots of partially resistant cv.

Discussion

Interactions between plants and nematodes, including sedentary nematodes are well documented (Endo & Wergin, 1973; Endo, 1991; Wyss, 1992; Meijer, 1993; Luzzi *et al.*, 1994; Pedrosa *et al.*, 1994; Sijmons *et al.*, 1994). But little is known about plant defense mechanisms induced by migratory endoparasitic nematodes, including the burrowing nematode *R. similis*.

The present ultrastructural investigation revealed that progression of the migratory nematode within the infected root is associated with severe modifications in host cell organization. The collapse of host cells located close to the nematode may be a consequence of phytotoxic secretions by the pathogen. In this respect, Hussey (1989) noted that most pathological disturbances induced by plant parasitic nematodes should be considered as a direct consequence of secretions injected into the host cells. Lysis of banana cells by *R. similis* may also result from nematode feeding. Accordingly, modifications of selected plant root cells into syncitium have been shown with *Heterodera glycines* (Goverse *et al.*, 1994). Also, *Trichodorus* spp. formed a feeding tube by rapid solidification of salivary secretions which extended through the perforation of the cell wall and permitted the withdrawal of organelles and cytosol into the mouth during food ingestion. Secretions from the dorsal gland were injected after perforation of the cell wall but before ingestion and they may predigest the cytoplasm (Sijmons *et al.*, 1994).

Middle lamella and cell wall breakdown observed close to tunnels caused by *R. similis* ingress within banana tissues was cytochemically confirmed using specific gold-probes for targeting pectin and cellulose polymers. Host cell wall degradation can be related to mechanical damages resulting from nematode movement. Our study also showed patterns of local enzymatic cell wall degradation raising the question of possible secretion of hydrolases by the pathogen. Such hydrolytic activity was investigated in *M. incognita,* but no cellulase activity was found in the oesophageal gland secretory granules (Sundermann & Hussey, 1988).

In our experiments, the presence of bacteria, always seen in the vicinity of the nematode but to a lesser extent in roots of the resistant cv., may possibly be responsible for the lytic degradation of host cell walls. The association of *R. similis* with other microorganisms during pathogenesis had already been described in field trials but never in controlled experiments. Blake (1966) indicated that banana roots inoculated with both *Fusarium oxysporum* and *R. similis* showed more extensive necrotic areas than when *R. similis* was inoculated alone. He suggested that root invasion by *R. similis* could be favoured by necrosis caused by the fungus which resulted in the weakening of host cell walls. In banana plantations, association between *R. similis* and the fungus *Cylindrocladium* sp. has also been reported leading to damage increase (Loridat, 1989). Riedel *et al.* (1985) tested interactions of the fungus *Verticillium dahliae* with *Pratylenchus scribneri,* the combination of which caused significant disease symptoms. Taheri *et al.* (1994) found a relation between the number of *P. neglectus* per plant

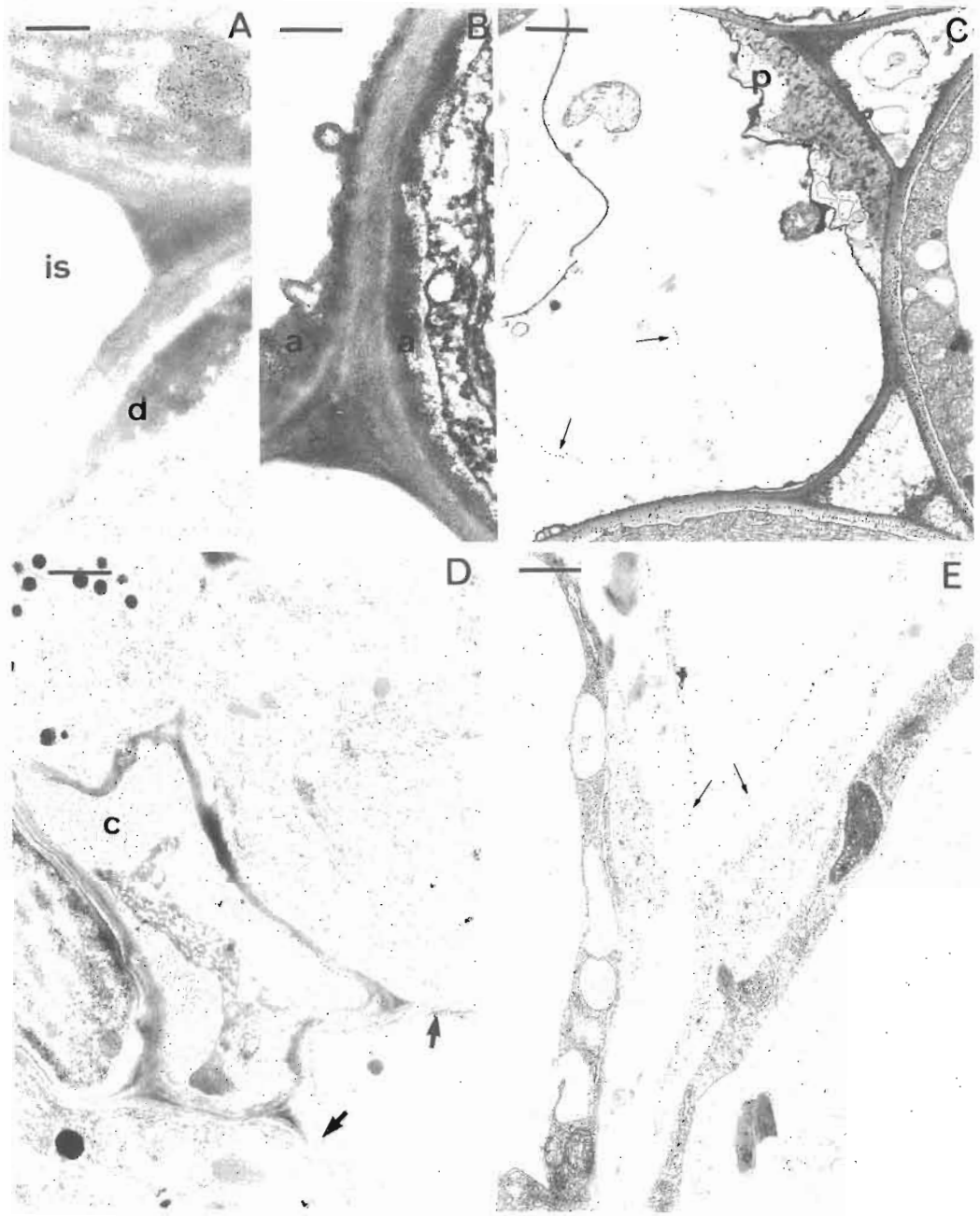

Fig. 6. *TEM micrographs. A : Cross section of an infected root of the susceptible cv., 4 days after inoculation; electron-dense material (d) is located in the paramural area of a phloem cell (is : intercellular space;* bar = 0.36 µm*); B : Cross section of an infected root of the susceptible cv. showing appositions (a) in the paramural areas of degraded phloem cells, 4 days after inoculation* (Bar = 0.36 µm); *C : Cytolocalization of ß 1, 4-glucans by means of a gold-complexed exoglucanase; this micrograph of an infected root of the susceptible cv. shows the presence of ß 1, 4-glucans located in an altered cell (arrows), 4 days after inoculation; a weak labelling is seen over the papilla (p) in the paramural area* (Bar = 0.86 µm); *D-E : Immunocytolocalization of ß-1,3-glucans using anti-ß-1,3-D glucopyranose polyclonal antibodies; D : The presence of callose pads (c) is observed in a degraded phloem cell of an infected root of the partially resistant cv., 4 days after inoculation; layers of callose are also observed in the adjacent cells (arrows;* Bar = 1 µm*); E : Cross section through a healthy root of the partially resistant cv.; a weak but even labelling is observed over parenchyma cell walls (arrows;* Bar = 0.5 µm*).*

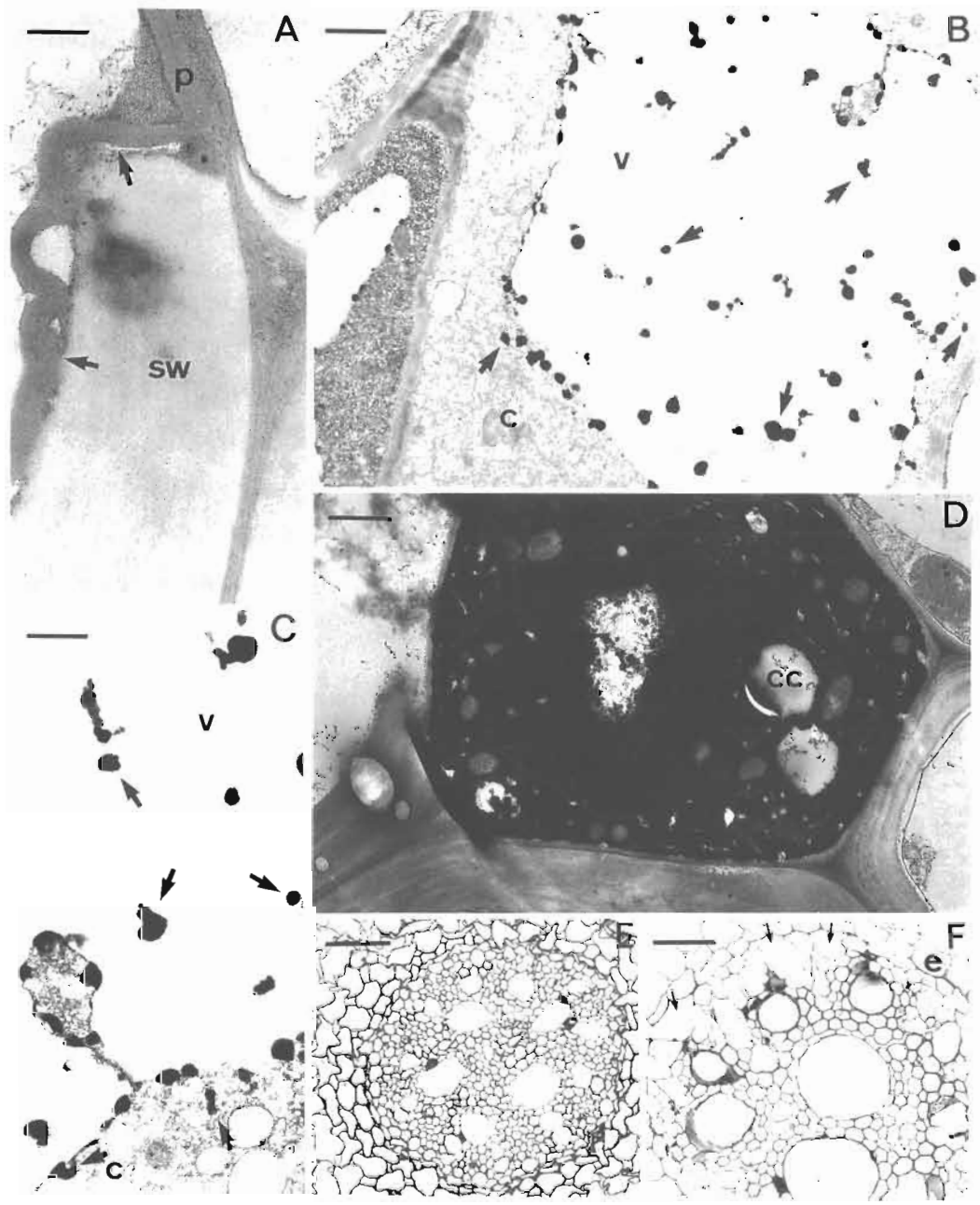

Fig. 7. *TEM observations of an infected root of the partially resistant cv. A : An electron-dense layer (arrows) coats the secondary cell wall (sw) and the pit area (p) of a xylem vessel, 4 days after inoculation* (Bar = 0.36 µm); *B-C : Electron-dense granules (arrows) are visible in the vacuole (v) of a cortical cell, 2 days after inoculation; some are also observed in the cytoplasm (c)* (Bars : B = 1.4 µm; C = 0.7 µm); *D : Vessel contact parenchyma cell (cc) showing a highly electron-dense cytoplasm, 4 days after inoculation* (Bar = 0.7 µm); *E-F : Sections through healthy (E) and infected (F) roots of the susceptible cv. 4 days after inoculation. Light microscope observations. Close to the endodermis (e) of an infected root (F), cells of the vascular stele are considerably larger (arrows) as compared to those in a healthy root (E)* (Bars : E, F = 12.5 µm).

Table 2. *Number of phenol-producing cells in healthy banana roots.*

	Plants age (weeks)			
Cultivar	2	4	6	8
Susceptible	17 ± 2	9 ± 3	15 ± 2	11 ± 1
Resistant	4 ± 1	2 ± 2	4 ± 2	6 ± 1

Data expressed as means from twenty roots.

and the severity of root lesions when the nematode was associated with the fungus *Rhizoctonia solani.* In line with these observations, the occurrence of bacteria in roots of infested banana by *R. similis* raises the question of the origin of these associated microorganisms; their identification and possible involvement in this interaction are currently underway.

The lower rate of nematodes encountered in infected roots of the resistant cv. confirms previous studies that suggests the existence of some constitutive mechanisms in the partially resistant cv. that may limit root penetration by *R. similis* (Sarah *et al.*, 1992). The secretion of nematode-repulsive molecules from plants (unpubl.) or the thickness of root peridermis are hypotheses that may explain such a limitation. Mace (1963) reported the presence of phenol producing-cells randomly distributed in paravascular zone of parenchyma tissues of healthy banana roots. In our experiment, we found that the number of phenol containing cells was higher, and the distribution different, in the healthy susceptible cv. than in healthy partially resistant plants. In light of these observations, we suggest that phenols in healthy roots probably do not contribute to the constitutive resistance of banana to *R. similis.* Studying plant-Heteroderidae interactions, Giebel (1974) showed that the level of resistance depended on the ratio of monophenols to polyphenols and not on the whole phenol concentration of host tissue. In contrast, the presence of ß-1,3-glucans in root cell walls of healthy resistant bananas as compared to roots of the healthy susceptible cv. indicates that these saccharides, which are involved in the reinforcement of walls, may interfere with nematode progression or feeding. It would be interesting to investigate the role of other molecules that play similar roles in cell wall reinforcement such as extensin, which is able to immobilize certain plant pathogens (Showalter, 1993).

Conversely to previous histological works (Blake, 1961), *R. similis* was found both in cortical and vascular cells of roots of the susceptible banana cv. In contrast, in roots of the resistant banana cv., the pathogen was located within the cortical cells only, suggesting that the endodermis acts as an effective barrier to nematode progression towards the vascular stele. Similarly, Mateille (1994) noticed that host tissue necrosis was less extensive in roots of the cv. Gros Michel as compared to the less resistant cv. Poyo. This author also reported that lesions induced by *R. similis* in Poyo roots extended through the internal cortical parenchyma and reached the vascular cylinder, while they remained in the medial layer of the cortical parenchyma in Gros Michel roots.

Histochemical tests shown that root endodermis cells of the partially resistant cv. were coated by a thick suberized layer which was not detected in roots of the susceptible cv. (unpubl.). In this cultivar, cell hyperplasia may be the only factor limiting the nematode progression in the vascular stele. In other plant-nematode interactions, endodermis was also demonstrated to be an efficient constitutive barrier to nematode ingress (Veech, 1978, 1979). In cotton roots infected by *M. incognita,* Veech showed that terpenoid aldehyde synthesis was induced more rapidly and extensively in endodermis cells of the resistant germplasm than of the susceptible one.

Several induced responses associated with root colonization were ultrastructurally observed in the infected banana plants. Most of them consisted in host cell wall modifications such as apposition layers where ß-1,3-glucans accumulated. The increase of callose synthesis is a common plant response to infection elicited by various pathogens. In *Criconemella xenoplax*-infected plants, callose was detected around stylets between the invaginated plasma membrane and the plant cell wall in feeding relationships (Hussey *et al.*, 1992). To our knowledge the present article is the first report of callose deposition in plant infected with migratory nematode. However, similar patterns observed in the immunolocalization of ß-1,3-glucans in roots of both susceptible and partially resistant infected banana cvs, indicate that callose synthesis was probably induced by mechanical stresses resulting from host cell wall breakdown, rather than constituing an effective barrier associated with pathogenesis.

The accumulation of electron-dense compounds after infection observed in roots of the partially resistant cv. only seemed to be an early feature in the host defense strategy. They were detected in paramural areas and in vacuoles of cortical parenchymal and phloem cells. Reactivity of these compounds to osmium tetroxide and toluidine blue indicates that these newly synthesized compounds could be from phenolic origin (Scalet *et al.*, 1989). Phenol compounds such as quinones have been recently reported as exhibiting nematicidal activity against *M. incognita* (Mahajan *et al.*, 1992). In this respect, we suggest that banana plants respond to *R. similis* by the activation of phenol synthesis that may contribute to resistance to the pathogen. Accordingly, Mace and Solit (1966) and Beckman and Mueller (1970) found that phenols, including 3-hydroxytyramine (dopamine) were involved in banana defense against *Fusarium,* a vascular pathogenic fungus.

In conclusion, the present microscopic investigation showed that *R. similis* colonizing banana roots causes severe alterations of host cells. Support for host cell wall

degradation was provided by cytochemistry which revealed that cellulose and pectin are altered during the pathogen ingress. An association of *R. similis* with bacteria was also demonstrated, although the possible role of this microorganism has to be established. Structural features of plant defense responses to the infection were also cytologically demonstrated, mainly concerning callose and phenol accumulation. Further investigation should deal with phenol histochemistry in order to identify compounds that may be involved in banana resistance to *R. similis* root rot.

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