Histological changes induced by *Rotylenchulus borealis* on corn and sweet potato and by *R. parvus* on sugarcane

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SUMMARY

Histological examination of corn *(Zea mays)* and sweet potato *(Ipomea batatas)* roots infected by *Rotylenchulus borealis* indicated two different cell responses of the two hosts. *R. borealis* established a permanent feeding site in an endodermal cell in corn roots, but in a cortical cell in sweet potato roots. In both hosts *R. borealis* induced syncytia formation that extended into the stele involving all the stelar tissues except metaxylum elements. A greater number of cells was involved in *R. borealis* syncytium of sweet potato than corn roots. Cross sections of sugarcane *(Saccharum officinalis)* infected by *R. parvus* showed that the nematode female established a permanent feeding site in the endodermis with consequent fusion of endodermal and pericyclic cells and syncytium formation.

RÉSUMÉ

Modifications histologiques produites par Rotylenchulus borealis *sur maïs et patate douce et par* R. parvus *sur canne à sucre*

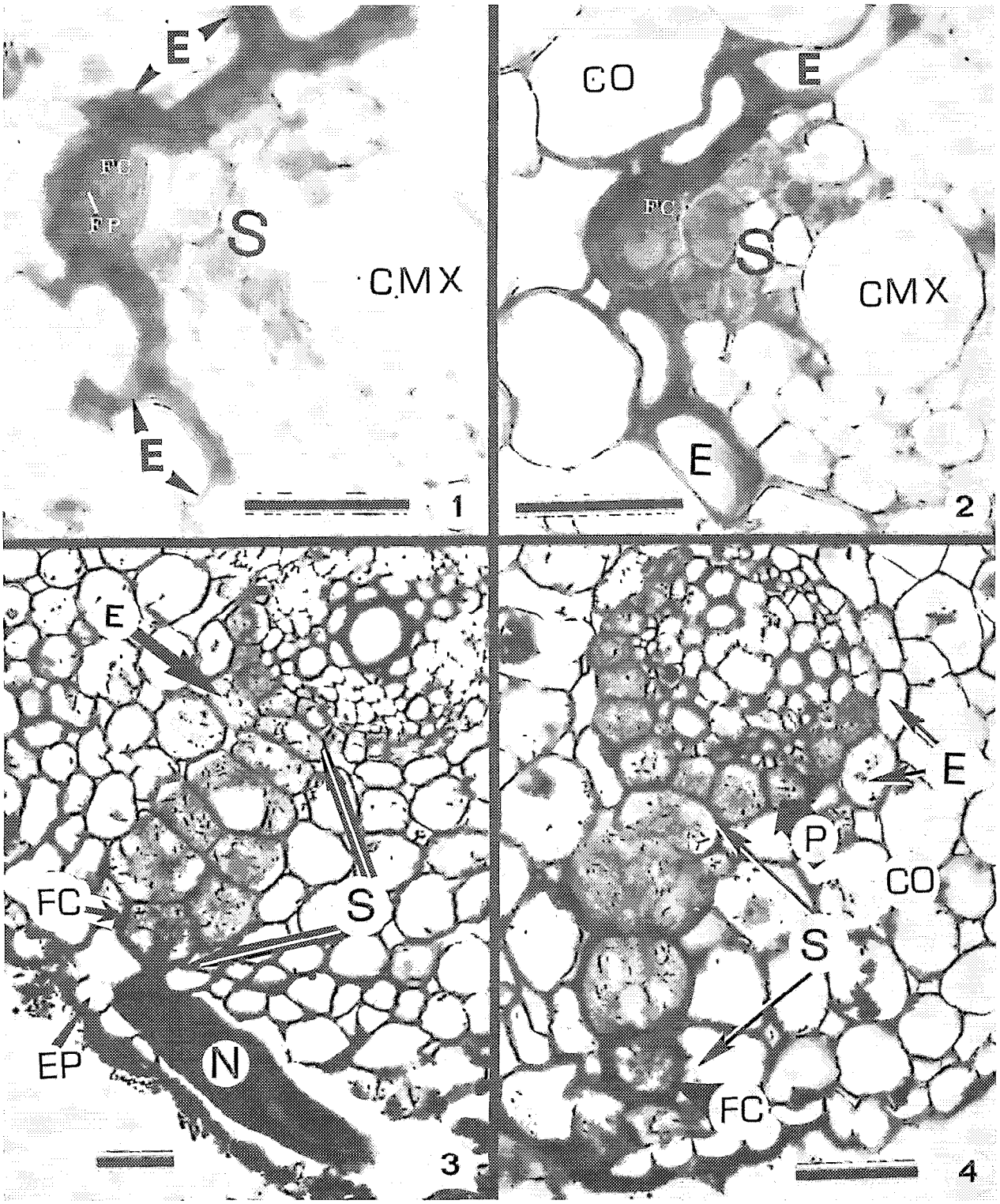
L'examen histologique de racines de maïs *(Zea mays)* et de patate douce *(Ipomea batatas)* infestées par *Rotylenchulus borealis*, a montré une réponse différente des deux hôtes au niveau cellulaire. Chez le maïs, *R. borealis* établit un site permanent d'alimentation dans une cellule de l'endoderme radiculaire, tandis que chez la patate douce il s'agit d'une cellule corticale. Chez les deux hôtes, *R. borealis* induit la formation de syncytia qui s'étendent dans la stèle, affectant l'ensemble des tissus du cylindre central, sauf les éléments du métaxylème. Un plus grand nombre de cellules participe à la formation des syncytia dans les racines de patate douce que dans celle de maïs. Les coupes transversales de racines de canne à sucre *(Saccharum officinalis)* infestées par *R. parvus* montrent que la femelle du nématode établit un site permanent d'alimentation dans l'endoderme avec pour conséquence la fusion de cellules de l'endoderme et du péricycle, et la formation d'un syncytium.

Different histopathological responses are caused by *Rotylenchulus* species in the roots of their hosts. *R. macrodoratus* Dasgupta, Raski & Sher usually forms an uninucleate giant cell originated from an endodermal cell, whereas *R. reniformis* Linford & Oliveira induces the formation of a syncytium also originated from endodermal cells in its host roots (Cohn, 1973; Cohn & Mordechai, 1977; Inserra & Vovlas, 1980). Variations in the response of the root cells to *R. reniformis* with syncytium formation from a pericyclic cell has been reported in different hosts (Razak & Evans, 1976). Uninucleate giant cells originating from a cortical cell and extending from the cortex into the stele has also been observed in thick roots infected by *R. macrodoratus* (Inserra & Vovlas, 1980). Root cell responses of these two parasites appeared to be influenced by the host. *R. borealis* Loof & Oostenbrink induces anatomical alterations in corn (*Zea mays* L.) roots that are similar to those of *R. reniformis* on cantaloupe (*Cucumis melo* L.), cotton (*Gossypium hirsutum* L.), soybean (*Glycine max* L.) Merr. and sunflower (*Helianthus annuus* L.) (Cohn, 1973; Heald, 1975; Robinson & Orr, 1980), but there are no other reports on the histological changes induced by *R. borealis* on other hosts (Vovlas & Inserra, 1982). The histological changes induced by *R. parvus* (Williams) Sher in infected roots are also unknown.

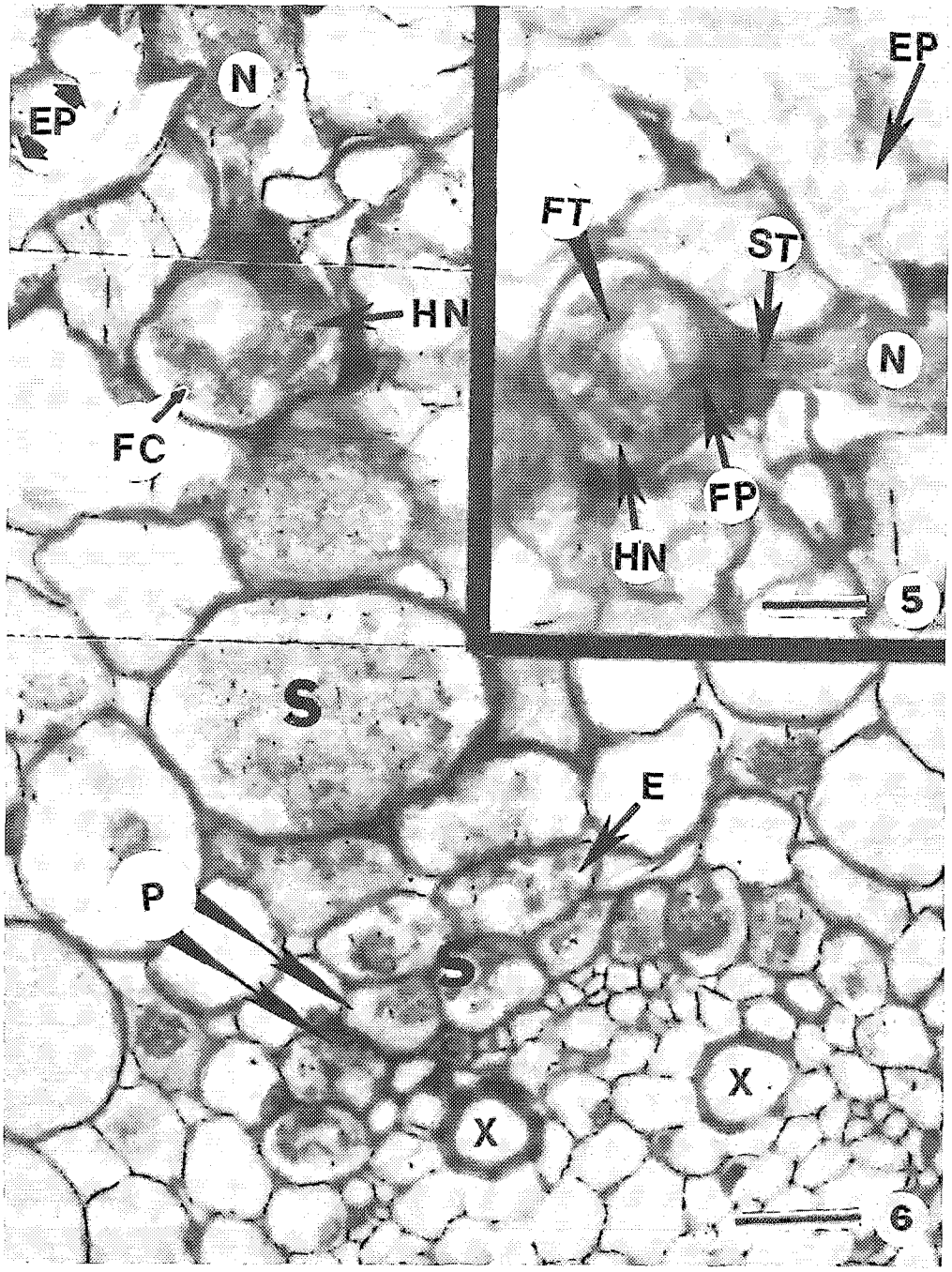
In this paper the cell responses of corn to *R. borealis* infection are compared to those observed in nematode infected sweet potato (*Ipomea batatas* L.) and the anatomical alterations induced by *R. parvus* in sugarcane *(Saccharum officinalis)* roots are illustrated.

Materials and methods

A *Rotylenchulus borealis* population from Rauscedo, North Italy, was used in our experiments. Sweet potato cuttings and pregerminated corn seeds were transplanted into a bin containing sandy clay loam soil (22.62 % clay, 10.76 % silt, 62.20 % sand, and 4.42 % gravel) infested with 0.5 *R. borealis* second stage juveniles (J2) and young females/cm³ soil and grown at 22° ± 4° in a greenhouse. Twenty eight days after transplantig ten seedlings per each host were harvested and the root systems gently washed free of soil. Corn and sweet potato feeder roots with nematode females protruding with the body posterior portion from the root surface were selected for histological examination under a ste-



Figures 1-4. Anatomical changes of corn (Figs 1& 2) and sweet potato (Figs 3& 4) infected with *Rotylenchulus borealis.*
Fig. 1 : Root cross section showing a syncytium (S) of *R. borealis* originated from an endodermal (E) cell and expanded into the stele. Note a feeding peg (FP) and thickened wall in the outer portion of the feeding cell (FC) wall. The wall thickening of the feeding cell (FC) in contact with the pericycle is degraded.
Fig. 2 : Root cross section showing a syncytium (S) of *R. borealis,* originated from an endodermal (E) cell and expanding into the stele. Feeding cell (FC), pericyclic, phloem, protoxylum, and vascular parenchymal elements show dense cytoplasm, CO = cortex, CMX = central metaxylem.
Fig. 3 : Root cross section showing a nematode (N) feeding on a syncytium (S) originated from a cortical (CO) cell and expanded into the stele. Note the funnel-shape of the syncytium. EP = epidermis, FC = feeding cell.
Fig. 4 : Root cross section showing a funnel-like syncytium (S) originated from a cortical (CO) cell and expanding into the stele. Note the hypertrophy of cortical, endodermal (E), and pericyclic (P) cells involved in the syncytium formation. Wall thickenings are visible in the syncytial cells of the cortex. FC = feeding cell.
(Scale bars = 25 µm in Figs 1 & 2; 50 µm in Figs 3 & 4).

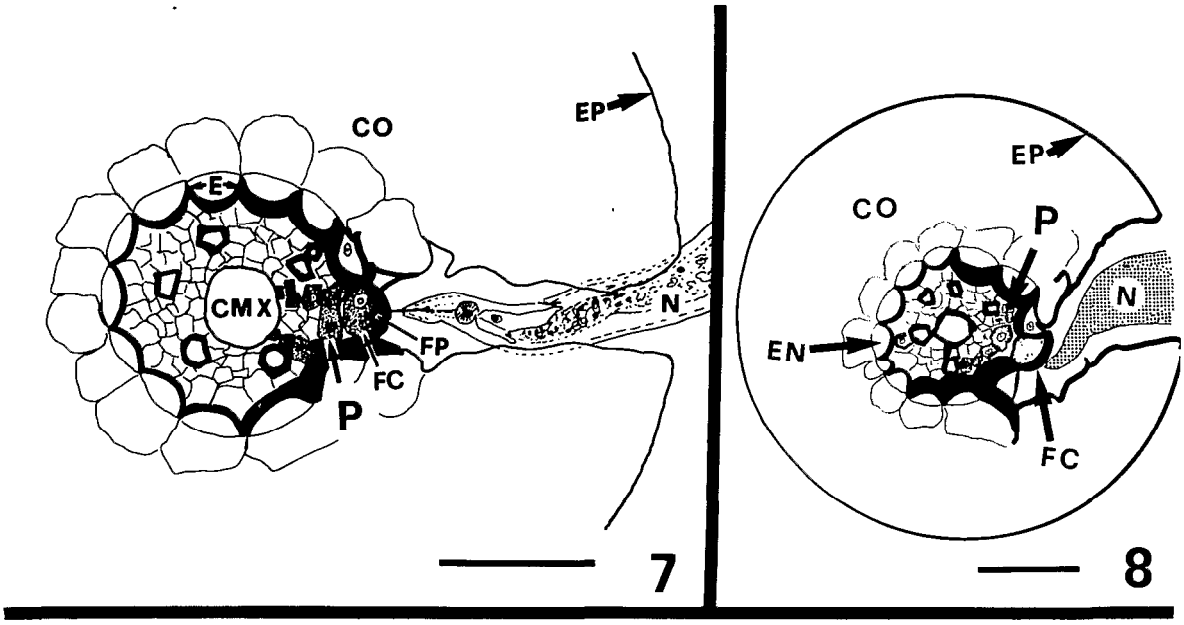


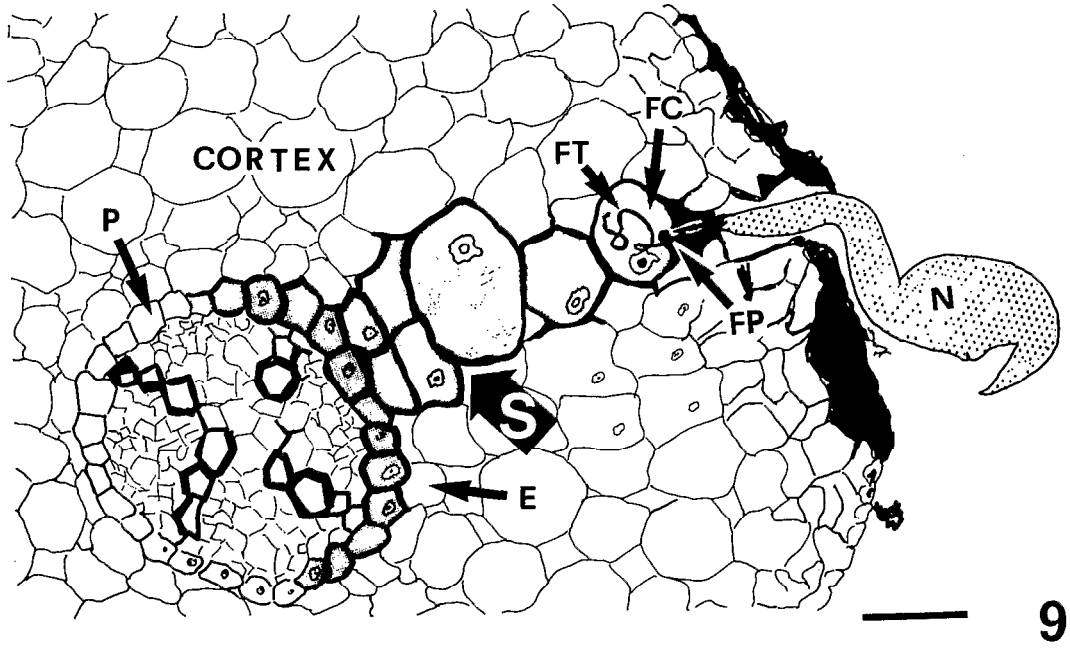
Figures 5-6 Sweet potato root infected with *Rotylenchulus borealis.*

Fig. 5 : Root cross section showing a nematode (N) with stylet (ST) inserted in a feeding cell and surrounded by a feeding peg (FP). A feeding tube (FT) is visible in the cytoplasm of the feeding cell; EP = epidermis, HN = hypertrophic nucleus.

Fig. 6 : Root cross section showing a nematode (N) feeding on funnel-like syncytium (S) that originated from a cortical cell and expanded into the stele. Note hypertrophic syncytial cells of cortex, endodermis (E) and pericycle (P); EP = epidermis, FC = feeding cell, HN = hypertrophic nucleus, X = xylem.

(Scale bar = 25 µm).





Figures 7-9. Composite camera lucida drawings, from serial sections of *Rotylenchulus borealis* feeding area in corn roots (7-8) and a sweet potato root (9), CMX = central metaxylem, CO = cortex, EN = endodermis, EP = epidermis, FC = feeding cell, FP = feeding peg, FT = feeding tube, N = nematode, P = pericycle.
(Scale bar = 50 µm).

reomicroscope. Infected roots were cut in 4-5 mm long segments, fixed in FAA, dehydrated in tertiary butyl alcohol and embedded in paraffin. The fixed root samples were sectioned at 10-15 µm, stained with safranin and fast-green, mounted in Dammar xylene and examined under a compound microscope (Johansen, 1940).

Rotylenchulus parvus infected sugarcane root obtained from Mount Edgecombe Experiment Station, South Africa, were stained with acid fuchsin in boiling lactophenol, sectioned free-hand with a razor blade, and mounted in clear lactophenol. Sections were studied and photographed under a compound microscope.

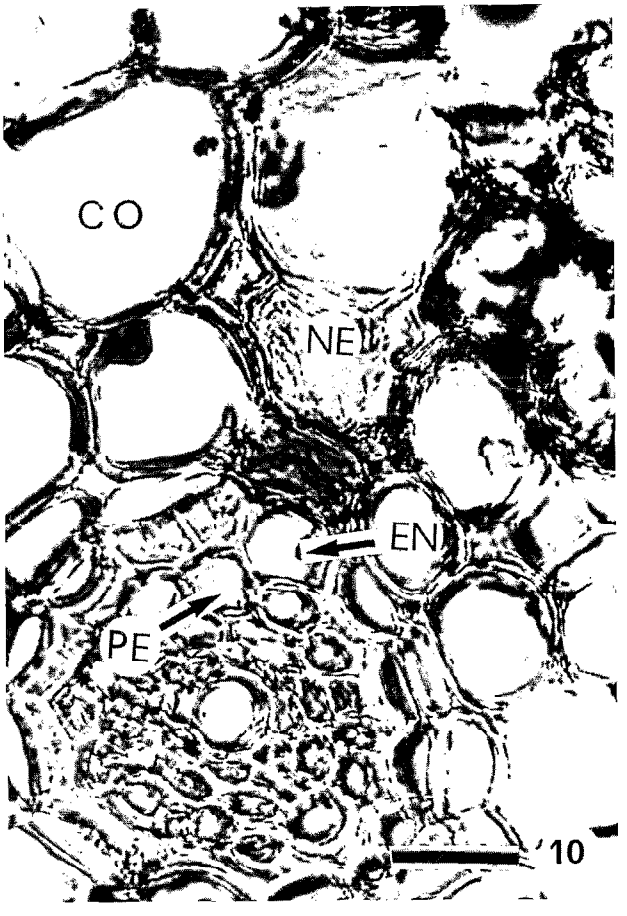


Fig. 10 Cross section of sugarcane root infected with *Rotylenchulus parvus*. The nematode female (N) has penetrated into the cortex (CO) establishing a permanent feeding site in the endodermis (EN). The endodermal cell wall thickening adjacent to pericyclic (PE) cell is degraded at nematode feeding site indicating fusion of the two cells and syncytium initiation.
(Scale bar = 20 µm).

Results and discussion

Histological examinations of *R. borealis* infected corn roots indicated that nematode females penetrated into the cortex with the anterior portion of the body and established a permanent feeding site in an endodermal cell (Fig. 1). This cell fused with adjacent pericyclic cells forming a syncytium, during the fusion the cell wall thickening in contact with pericycle degraded (Figs 1, 2). Thickening was observed instead along the wall of this cell at the point of stylet penetration, where a feeding peg surrounding the inserted nematode stylet could be discerned protruding into the cell (Figs 1, 2). In addition to pericyclic cells, also phloem, protoxylum and vascular parenchymal cells were involved in the syncytium expansion (Fig. 2). All these syncytial cells showed very dense cytoplasm and more or less accentuated wall fragmentation (Figs 1, 2). These observations confirmed previous host responses reported for corn to *R. borealis* (Vovlas & Inserra, 1982).

Observations of cross sections of *R. borealis* infected sweet potato roots indicated that the nematode females penetrated only into two ot three layers of cortical cells without reaching the stele (Figs 3-5). In these roots the nematode established a permanent feeding site in a cortical cell that fused with adjacent cells, forming a syncytium that expanded into the stele by subsequent fusions with endodermal and pericyclic cells. Structurally, this syncytium was somewhat similar to the « bridgehead » configuration formed by *R. reniformis* in cowpea root, as described and illustrated by Razak and Evans (1976 : Fig. 3).

About 30-80 cells of cortical parenchyma, 10-20 cells of endodermis, and 100 cells of pericycle formed this syncytium, which in cross section showed a funnel-like shape with the enlarged portion into the stele and the narrow and elongated base into the cortex (Figs 3, 4,6). Cortical, endodermal, and pericyclic cells in the syncytium were hypertrophic, with dense cytoplasm and enlarged nuclei (Fig. 6). Also protoxylum, phloem, and vascular parenchymal cells were involved in the syncytium enlargement (Figs. 4, 6). Accentuated wall thickenings were observed in the feeding cell and also in the other syncytial cells of the cortex (Figs 3-6). A feeding peg formed by cell wall material surrounding the stylet penetrated only into two or three layers of cortical cells tube 2 µm wide and 80-90 µm long extended from the feeding peg into the cytoplasm of the feeding cell (Fig. 5).

From these results it appeared that two different cell responses to *R. borealis* occurred in infected corn and sweet potato roots. *R. borealis* established a feeding site in an endodermal cell in corn roots, but in a cortical cell in sweet potato roots (Figs 7-9). In both hosts the nematode induced syncytia formation that expanded into the stele involving pericyclic, phloem, protoxylum and vascular parenchymal cells, but not metaxylum

elements. The syncytium of *R. borealis* in sweet potato roots involved a much greater number of cells than the syncytium in corn roots. A feeding peg was observed in the feeding cells of both syncytia; however a feeding tube was detected only in the syncytium of sweet potato roots (Figs 7-9).

These findings indicated that feeding peg and feeding tube are common characteristics of the general feeding process of *Rotylenchulus* species. There is ample evidence of their presence in feeding cells of *R. reniformis* (Razak & Evans, 1976) and also of *R. macrodoratus* : (Cohn & Mordechai, 1977 : Fig. 6). Also for *R. borealis* as for *R. macrodoratus* and *R. reniformis* the root cell responses were greatly influenced by the host.

Cross sections of *R. parvus* infected sugarcane roots showed that female nematodes penetrated into the cortical parenchyma and came to rest in the endodermis (Fig. 10). Endodermal cell walls were deeply stained with acid fuchsin at the nematode feeding site and the cell wall thickening in contact with pericycle was degrated, indicating fusion process between endodermal and pericyclic cells with syncytium initiation (Fig. 10). The root cell responses induced by *R. parvus* on sugarcane were similar to those of *R. borealis* on corn (Vovlas & Inserra, 1982) and to those of *R. reniformis* on cotton, cantaloupe, and sunflower (Cohn, 1973; Heald, 1975; Robinson & Orr, 1980).

The results of these studies indicate that two types of plant reaction are induced by *Rotylenchulus* species : *i)* an uninucleate giant cell originated usually from the endodermis induced by *R. macrodoratus*, and *ii)* a syncytium originated also from endodermis induced by *R. borealis*, *R. parvus*, and *R. reniformis*.

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