Fundam. appl. Nematol., 1992, **15** (1), 63-70.

Comparison of host response of *Ekphymatodera thomasoni* with other Heteroderinae

Manuel MUNDO-OCAMPO and James G. BALDWIN

Department of Nematology, University of California, Riverside, CA 92521, USA.

Accepted for publication 1 February 1991.

Summary — *Ekphymatodera thomasoni* Baldwin, Bernard & Mundo-Ocampo, 1989 induces a poorly defined syncytium on *Carex* and *Juncus* hosts which is distinctive from that of all other Heteroderinae examined. The syncytium is small with a greatly thickened wall near the lip region of the nematode. Wall ingrowths are absent, nuclei are large and irregular with multiple nucleoli, and there is no evidence of proliferation of nuclei within the syncytium. The unusual syncytium is morphologically intermediate between the thick-walled single uninucleate giant cell of Sarisoderini and the large multinucleate syncytium lacking wall ingrowths in Ataloderini. The phylogenetic significance of the host response of *E. thomasoni* must be interpreted in light of a large matrix of additional characters.

Résumé — ***Réactions de l'hôte à* Ekphymatodera thomasoni *et comparaison avec celles aux autres Heteroderinae*** — *Ekphymatodera thomasoni* Baldwin, Edward & Mundo-Ocampo, 1989 induit chez les plantes hôtes *Carex* et *Juncus* un syncytium pauvrement différencié, très différent de tous ceux observés chez les autres Heteroderinae. Ce syncytium est peu étendu, la paroi en étant très épaissie au voisinage de la région labiale du nématode. Les apophyses internes de la paroi sont absentes, les noyaux bien développés et comportant de nombreux nucléoles irréguliers; il n'y a aucune évidence d'une prolifération de ces noyaux. Ce syncytium inhabituel est morphologiquement intermédiaire entre, d'une part la cellule géante uninuclée et pourvue d'une paroi épaisse des Sarisoderini et, d'autre part, le grand syncytium à paroi dépourvue d'apophyses internes des Ataloderini. La signification de la réaction de l'hôte à *E. thomasoni* doit être interprétée à la lumière d'une matrice étendue de caractères supplémentaires.

Key-words : *Ekphymatodera*, Heteroderinae, host response, giant cells.

Sedentary endoparasitic nematodes, including Heteroderinae, Meloidogyninae, Nacobbinae, *Rotylenchulus*, and *Tylenchulus* induce host nurse cells. These cells are specialized to sustain nutritional requirements for growth, development, and reproduction of the parasite. However, the structure of nurse cells is specific to each nematode taxa. A given nematode consistently induces the type of nurse cell characteristic of that nematode regardless of the host species (Jones & Dropkin, 1975; Jones, 1981; Mundo-Ocampo & Baldwin, 1983*a*, *b*, *c*, 1984). This specificity of host response is probably associated with nematode-specific characteristics of the stylet exudate which regulate particular host genes (Acedo *et al.*, 1984; Mundo-Ocampo & Baldwin, 1984, 1990; Burrows, 1990).

In the diverse subfamily Heteroderinae, two basic types of nurse cells have been described : the syncytium (SYN) and the single uninucleate giant cell (SUGC). The SYN in Heteroderinae is a mass of several enlarged cell units in which the cytoplasm is interconnected between discontinuous walls shared by the units (Fig. 1 A, C, D). The SYN varies among nematode taxa by the presence or absence of deep wall evaginations, wall ingrowths (Fig. 1 C; Table 1). Wall ingrowths increase the surface area of the cell in the region of transfer of solutes from adjacent vascular tissue (Jones, 1981). The SYN is induced by all members of Ataloderini and Heteroderini (Mundo-Ocampo & Baldwin, 1983*a*; Baldwin & Schouest, 1990) (Table 1).

The SUGC is characterized by a dramatic increase in size relative to non-nurse cells, a proportionally enlarged nucleus, and a greatly thickened cell wall in the region adjacent to the nematode's lip region (Fig. 1 B). This type of host response has been described in *Meloidodera*, and Sarisoderini (Mundo-Ocampo & Baldwin, 1983*b*, *c*, 1984; Baldwin & Schouest, 1990) (Table 1). Wall ingrowths never have been reported in a SUGC. Generally SYN or SUGC lacking wall ingrowths are connected with adjacent vascular tissue by abundant pits and pit fields (Mundo-Ocampo & Baldwin, 1983*a*, *b*).

Baldwin and Schouest (1990) recently treated the type of nurse cell as a character in a large matrix of features for a parsimonious computer-generated phylogenetic analysis of Heteroderinae. The type of nurse cell generally had a high degree of consistency with other characters. However, parallel evolution of the SYN was hypothesized on the basis of parsimony, for *Verutus* and a new genus, *Ekphymatodera* Baldwin *et al.*, 1989. *Ekphymatodera* was tentatively placed within Sarisoderini, a group which is otherwise characterized by a SUGC. The host response of *Ekphymatodera* was coded as a SYN, based on preliminary hand sections of living material. Detailed description of the nurse cell of *E. thomasoni* is needed to further consider this character in testing hypotheses of phylogeny of Heteroderinae.

ISSN 1164-5571/92/01/63 08 $ 2.40 / © Gauthier-Villars - ORSTOM

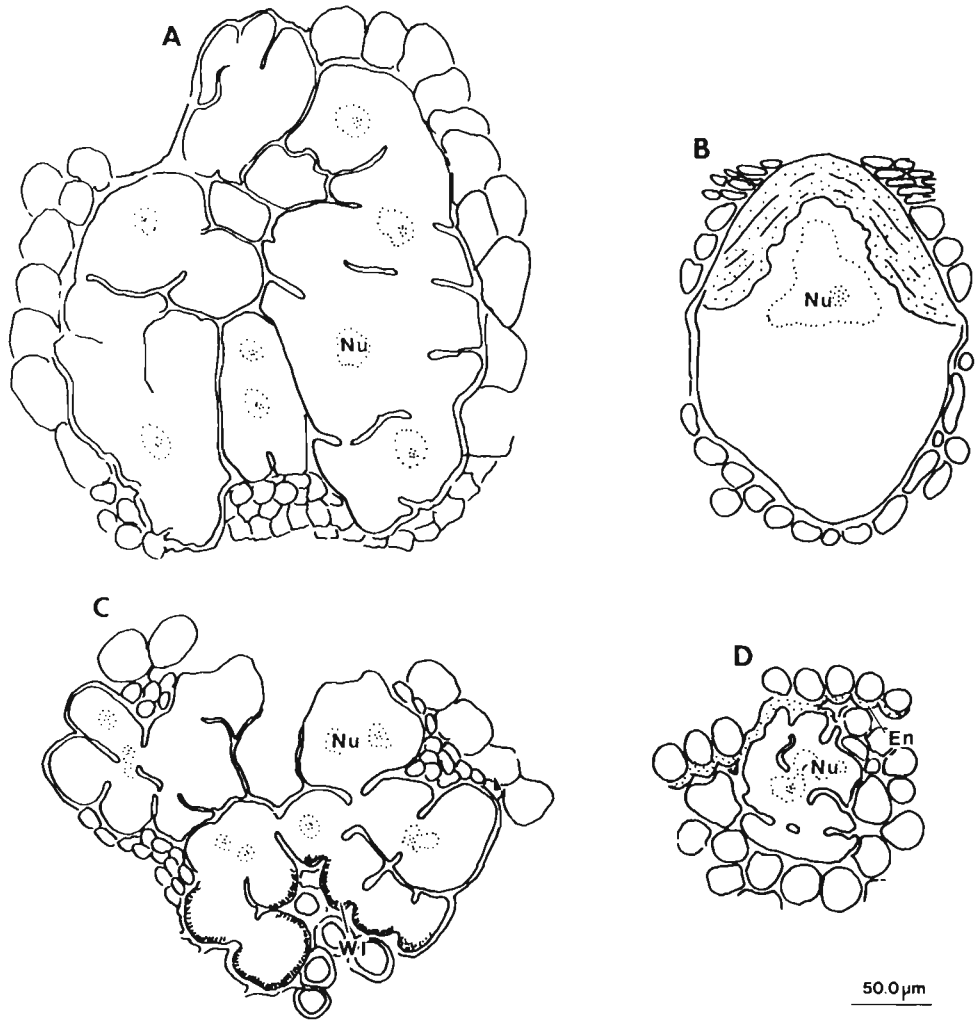

Fig. 1. Diagrammatic representation of host responses of Heteroderinae. Nurse cells oriented so that vascular tissue is toward bottom of page. A : Multinucleate syncytium of *Atalodera* lacking wall ingrowths; B : Single uninucleate giant cell of *Sarisodera;* C : Multinucleate syncytium of *Heterodera* with wall ingrowths (WI); D : Diminutive multinucleate syncytium of *Ekphymatodera thomasoni* lacking wall ingrowths (En = endodermis, Nu = nucleus).

Materials and methods

A culture of *E. thomasoni* was established on the type host *Juncus effusus* L. var. *pacificus,* as well as on *Carex* sp. L. in the greenhouse. Noninfected control roots as well as roots containing females at different levels of maturity, were selected, using a dissecting and inverted interference optical microscope. Small pieces of host roots with females were excised and prepared for histological examination, including bright field and interference light microscopy (LM), as well as scanning (SEM) and transmission (TEM) electron microscopy.

For LM and TEM, roots were fixed under light vacuum 4-6 h in Karnovski's (1965) fixative at 22 °C, rinsed, and postfixed in 2 % osmium tetroxide for twelve hours at 4 °C. Specimens were dehydrated in an ethanol series, infiltrated in Spurr's epoxy resin, and sectioned using glass knives. Sections for LM were 1-2 µm thick and examined with interference contrast unstained, or they were stained with aqueous 1.0 % toluidine blue (Mundo-Ocampo & Baldwin, 1984). Sections for TEM were cut about 0.09 µm thick, stained with uranyl acetate and lead citrate (Venable & Coggeshall, 1965) and examined on the Hitachi H 600 at 75 kV.

For SEM, small pieces of roots with females were fixed in 3.5 % glutaraldehyde, rinsed, and postfixed in 1 % osmium tetroxide for 12 h each at 4 °C. Dehydration

Table 1. Comparison of host response of selected Heteroderinae.

Genus	Nurse cell	Size in length (parallel to root axis)	Wall ingrowths	Authors
Heterodera	SYN	up to 2 mm	present	Endo, 1964; Jones, 1981
Globodera	SYN	up to 2 mm	present	Feldmesser, 1953
Cactodera	SYN	up to 2 mm	present	Baldwin & Bell, 1985
Punctodera	SYN	< 200 µm	uncertain	Suarez *et al.;* Mundo-O. & Baldwin (unpubl.)
Afenestrata	SYN	< 200 µm	absent	Baldwin & Bell, 1985
Atalodera	SYN	up to 1 mm	absent	Mundo-O. & Baldwin, 1983*a*
Ekphymatodera	SYN	< 200 µm	absent	Mundo-O. & Baldwin
Verutus	SYN	< 200 µm	uncertain	Cohn *et al.*, 1984; Mundo-O. & Baldwin (unpubl.)
Sarisodera	SUGC	< 300 µm	absent	Mundo-O. & Baldwin, 1983*c*
Meloidodera	SUGC	< 600 µm	absent	Mundo-O. & Baldwin, 1983*c*

was in ethanol. Specimens were critical point dried, mounted, coated with 20 nm gold-paladium, and observed on a JOEL 35 C SEM at 15 kV. All fixatives and rinse solutions for LM, TEM, and SEM, used .05 M phosphate buffer. At least seven samples were examined for each treatment.

Results

Ekphymatodera thomasoni induces a poorly defined SYN on *J. effusus* and *Carex* sp. which is distinctive from other Heteroderinae examined, with respect to its small size, lack of wall ingrowths, few nuclei, and thickened wall near the lip region of the nematode (Fig. 1 D). Second stage juveniles enter the tips of host roots shortly after hatching (Fig. 2 A). As a juvenile matures to a female and enlarges it eventually ruptures the root so that most of the body is external to the root (Figs 2 B, C). In some cases a portion of the root cortex is pushed outward by the body (Fig. 2 C), but no galling is present. Although a large egg mass is produced, it encloses only about 50 eggs; additional eggs are not retained in the body of the moribund female.

As a second stage juvenile invades the roots, surrounding cells may become necrotic and collapse. At the feeding site of developing juveniles a few cells of the endodermis, pericycle and adjacent vascular cells become hyperplastic and their cytoplasm becomes dense relative to noninfected cells (Figs 3 A, B, 4 A). As the cells enlarge, walls between them become discontinuous resulting in a minute SYN about 25 µm in transverse and longitudinal section (Fig. 3 B). As the nematode matures to an adult, the cells further enlarge, walls are increasingly fragmented, and the cytoplasm is reduced in density. The length and width of the SYN of the adult is about 60 µm (Fig. 3 C, D). The wall of the SYN is of uneven thickness, being thickest adjacent to the lip region. This thickened region, comprised of parallel fibrils, appears to be continuous with the thick wall of adjacent, apparently normal, endodermal cells (Fig. 3 A, C, 4 C). Distal from the lip region the walls of the SYN, including partial walls within the SYN, usually are not abnormally thickened (Fig. 3 C, D). Wall ingrowths are not induced by *E. thomasoni* (Fig. 4 F). Pit fields with plasmodesmata were not observed by TEM or SEM in the outer boundary of the SYN of *E. thomasoni.* The SYN is not abruptly distinguished from surrounding tissues. Cells adjacent to the SYN are not hyperplastic, there is no hypertrophy, and the dense cytoplasm of the SYN gradually diminishes with distance from the lip region (Fig. 3 D).

Each nucleus of the SYN of *E. thomasoni* is hypertrophied and distorted relative to that of a healthy cell; nevertheless the nuclear membranes are intact (Fig. 4 E, E'). The nucleus is large and diffuse and may include several nucleoli. We have not yet found more than a single large nucleus is present in each cell-like unit of the SYN. Frequently, nuclei occur in close proximity across cell gaps (Fig. 4 E). The cytoplasm is relatively dense and filled with small vacuoles, smooth endoplasmic reticulum, mitochondria, and various inclusions early in development of the syncytium (Fig. 4 B). However, in later stages the cytoplasm is less dense and only vacuoles and inclusions remain (Fig. 4 D). The SYN of *E. thomasoni* deteriorates soon after the female becomes sexually mature. Highly vacuolated, electron lucent SYN are associated with females which continue to lay eggs.

Discussion

Ekphymatodera thomasoni induces a unique nurse cell. The SYN is much smaller than that of Ataloderini or cyst-forming Heteroderini, except *Punctodera.* In size, the SYN of *E. thomasoni* resembles that of *Verutus* and *Afenestrata.* The absence of wall ingrowths is shared with *Atalodera, Afenestrata,* and genera having a SUGC. The thick nurse cell wall adjacent to the lip region of the nematode, and the large irregular nucleus in each cell

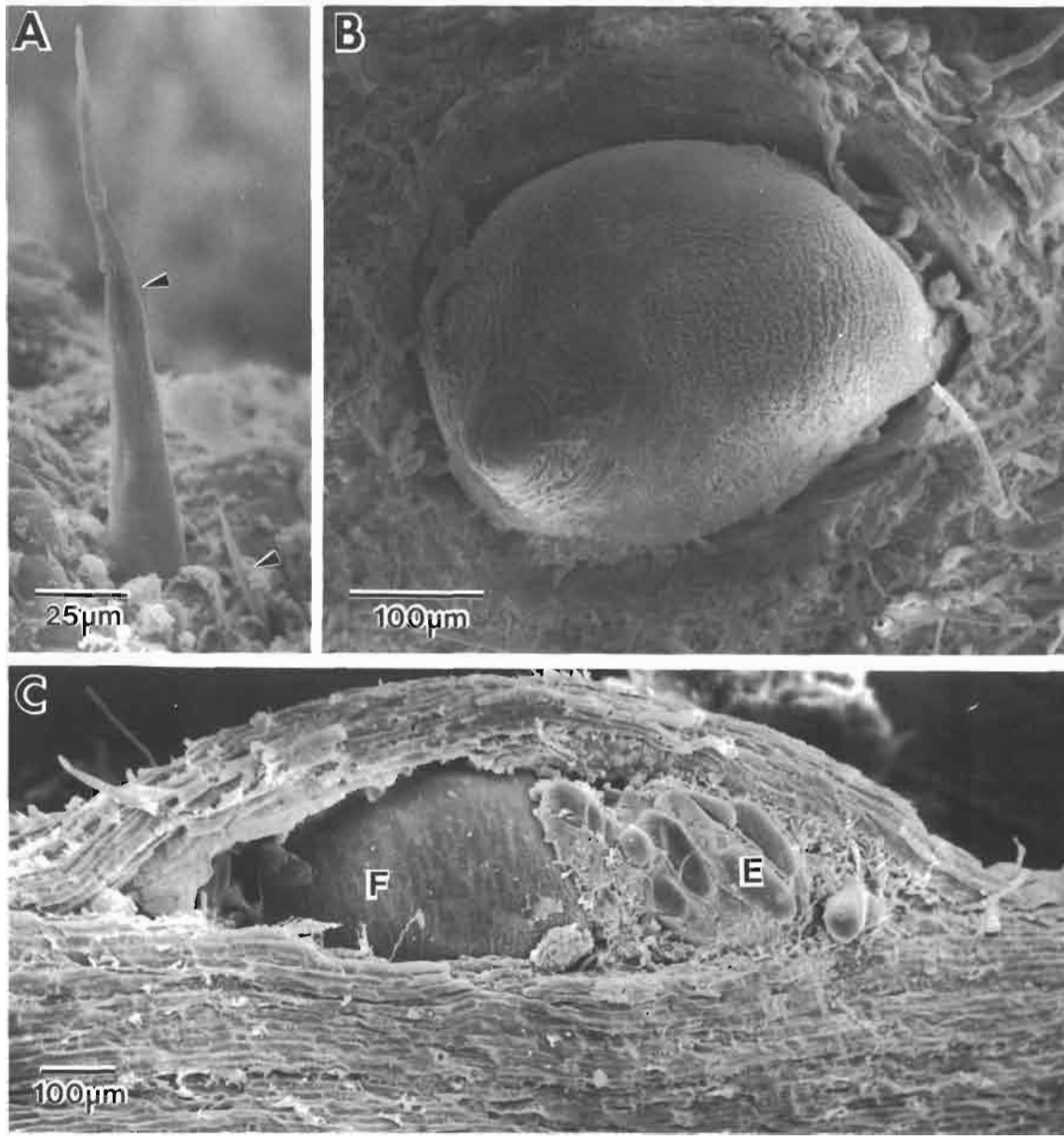

Fig. 2. SEM of *Ekphymatodera thomasoni* in relation to host roots. A: Second stage juveniles (arrows) penetrating root. One juvenile has nearly completed penetration with only the tail tip protruding; B : Mature female protruding from root; C : Mature female (F) emerging from under root cortex (E = eggs).

unit are similar to the SUGC of *Sarisodera*. These features of the SYN in *Ekphymatodera* may be significant in interpreting phylogenetic relationships of *Ekphymatodera*. However, parsimony arguments suggest that some aspects of the host response, including the SYN of *Verutus* and *Ekphymatodera* are convergent (Schouest & Baldwin, 1990). Although development of SYN or a SUGC is determined by the nematode rather than the host (Mundo-Ocampo & Baldwin, 1984), it is uncertain to what extent details such as size of the nurse cell and wall thickening might be affected by the host. Therefore, we tentatively coded host response for phylogenetic analysis of Heteroderinae as only two character states, SUGC and SYN, pending a more complete understanding of variation in nurse cells (Baldwin & Schouest, 1990).

Size of the nurse cell must be carefully considered as a potential character for phylogenetic analysis. The

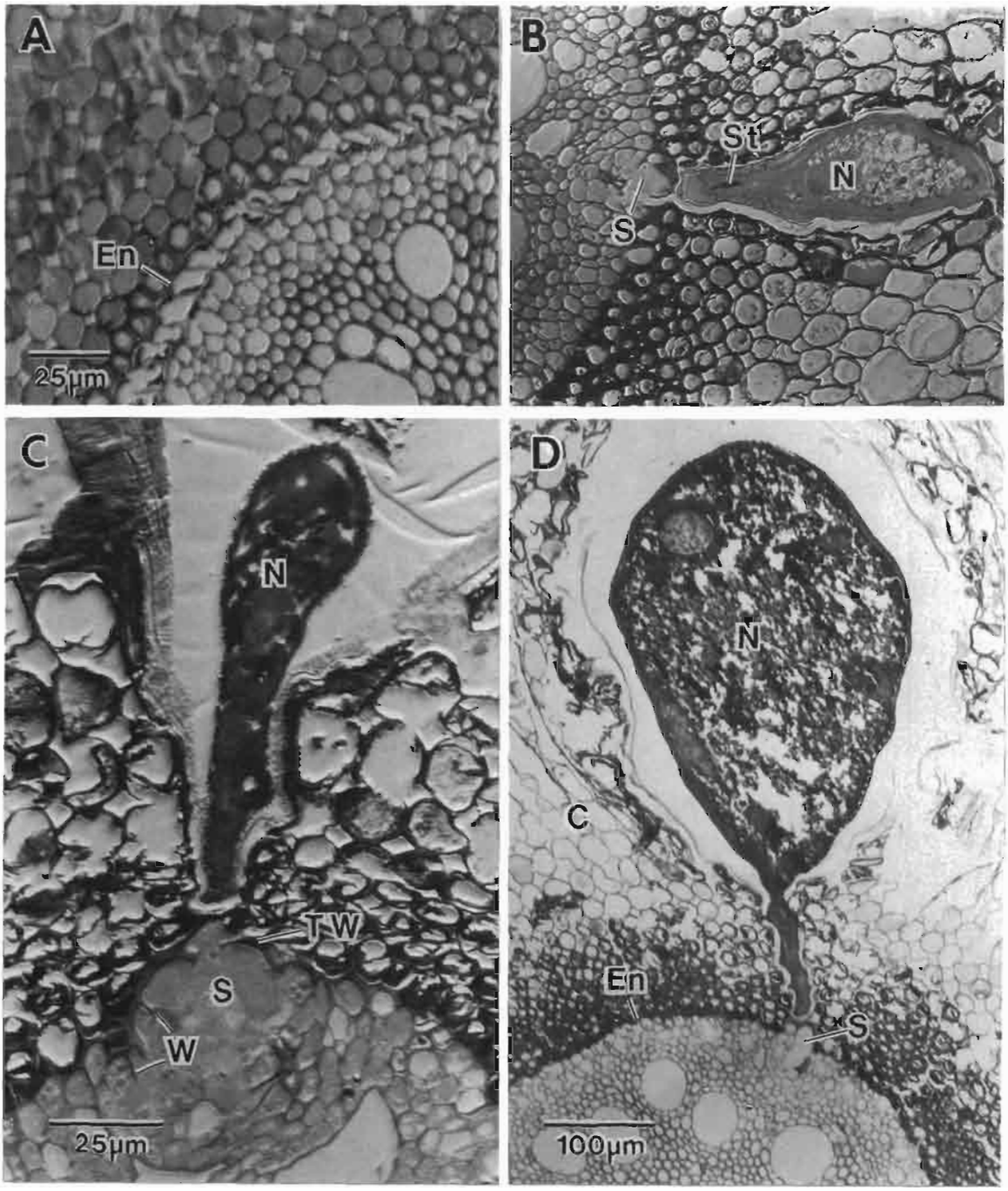

Fig. 3. Stained cross section of *Juncus effusus* viewed with light microscope interference optics. A : Noninfected root showing endodermis (En) boundary between vascular cylinder and cortex; B : Immature (third or fourth stage) *Ekphymatodera thomasoni* nematode (N) with developing syncytium (S) (St = stylet). Scale same as A; C : Head region of female nematode (N) of *E. thomasoni* associated with the thickened wall (TW) of a fully developed syncytium (S) (W = cell wall fragments); D : A fully developed syncytium (S) in relation to a mature female nematode (N) (C = cortex, En = endodermis).

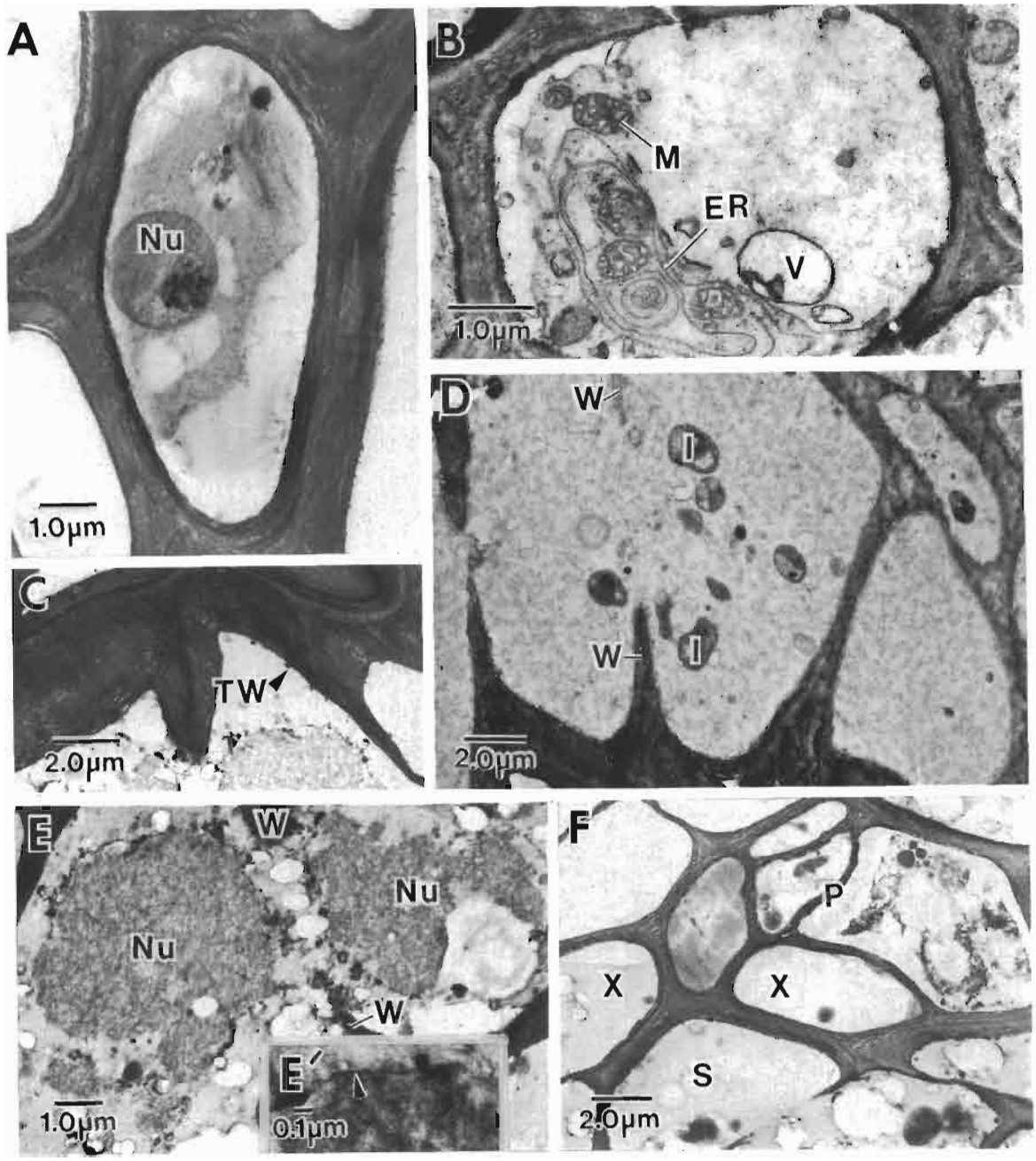

Fig. 4. Cross sections of *Juncus effusus* viewed with TEM. A : Noninfected parenchyma cell with nucleus (Nu) near the endodermis within the vascular cylinder; B : Portion of developing syncytium associated with immature *Ekphymatodera thomasoni*. Cytoplasm dense with endoplasmic reticulum (ER), abnormal mitochondria (M), and vacuoles (V). This is portion of the same syncytium shown with light microscopy in Fig. 3 B; C : Thickened wall (TW), composed of parallel fibrils, near the site of stylet penetration of *E. thomasoni;* D : A portion of adjacent cell units of a syncytium associated with mature *E. thomasoni* (I = inclusions; W = cell wall fragments); E : Diffuse nuclei (Nu) of adjacent cell units of syncytium of *E. thomasoni;* E' : Enlargement from D showing nuclear membrane (arrow) (W = cell wall fragments); F = Cells of vascular cylinder at boundary of syncytium (S) of *E. thomasoni;* wall ingrowths are not present (P = abnormal phloem; X = xylem vessels).

maximum size of SYN of mature *E. thomasoni* is about 60 µm diameter and it barely disrupts the periphery of the host's vascular cylinder. In comparison, the SYN of *Heterodera*, for example, is small early in development but in mature females it may increase to nearly fill the vascular cylinder of the host, and the length may be more than 2 mm (Jones, 1981; Acedo *et al.*, 1984). The size difference may relate to the length of time the SYN is sustained by the nematode. In *Ekphymatodera* the SYN we examined reached maximum size in the young female before eggs were produced. During egg development and laying eggs, the SYN is nearly completely vacuolated and appears to be dead. *Ekphymatodera* and *Heterodera* clearly utilize different strategies for survival which differentially impact the host. In the former only about 50 eggs are quickly produced, in the latter hundreds of eggs may be produced during a longer period. *Heterodera* may place greater energy demands on the host, requiring a larger more persistent nurse cell, than *Ekphymatodera*.

Differences in size of the SYN may also be directly affected by the host. One could hypothesize that SYN are generally smaller on monocots than on dicots. The small SYN of *E. thomasoni* on *Juncus* and *Carex*, *Punctodera chalcoensis* on *Zea* (Suarez *et al.*, 1985), and *Afenestrata africana* on *Panicum* (Baldwin & Bell, 1985), might support this hypothesis if it could be established that these species induce a more robust SYN on dicots. Conversely, *Verutus volvingentis* on the dicot, *Diodium*, induces a very small SYN (Cohn *et al.*, 1984), and *Heterodera* spp. do not produce a notably diminutive SYN on monocots (Johnson & Fushtey, 1966; Vovlas, 1985). Clearly, size of the SYN is a complex character which could be influenced by the nematode, host, and/or environment, and must be treated cautiously for phylogenetic analysis.

In *E. thomasoni* TEM analysis has not revealed more than a single very large nucleus with several nucleoli per cell-like unit of the SYN. The large size of the nucleus relative to the cell-like unit resembles the size relationship of the nucleus to the SUGC of Sarisoderini. In this respect, the SYN of *E. thomasoni* appears to be morphologically intermediate between the SUGC and the large multinucleate SYN. Apparently, there is no proliferation of nuclei in the SYN of *E. thomasoni*. The significance of this finding must be interpreted by more careful consideration of nuclei of the nurse cells of other Heteroderinae including *Verutus* and *Afenestrata*. Changes in nuclei of developing SYN including karyokinesis have rarely been considered, even in economically important cyst-forming genera. However, some reports suggest that mitosis is not stimulated by *Heterodera* (Jones & Northcote, 1972). Consideration of numbers of nuclei is complex and generally requires serial sections and careful tracing of elaborate lobes of irregularly-shaped nuclei (e.g. Mundo-Ocampo & Baldwin, 1983*c*). Visualization of entire SYN by confocal microscopy may prove to be advantageous in evaluating possible karyokinesis in different types of Heteroderinae.

The SYN of *E. thomasoni* is unusual in that it has indistinct boundaries separating it from adjacent " normal " cells. In *E. thomasoni*, hypertrophy and dense cytoplasm only gradually diminish with distance from the lip region of the nematode and cell walls of the SYN, distal from the lip region, are not abnormally thickened. Walls adjacent to vascular tissue have no special adaptations such as abundant pit fields or wall ingrowths for transfer of solutes. Wall ingrowths are present in *Heterodera*, *Globodera*, and *Cactodera*, but absent in *Atalodera*. Although they have been reported in *Punctodera* (Suarez *et al.*, 1985) we suggest that they may have been confused with other structures and consider that further evaluation, specifically with TEM, is needed. Similarly, wall ingrowths are not reported for *Verutus* and *Afenestrata*. However, TEM is needed to confirm their absence because wall ingrowths are sometimes short and sparse so that they are not clearly resolved with the LM (Kim *et al.*, 1986). Melillo *et al.* (1990) reported absence of wall ingrowths in SYN induced by *Globodera pallida* in potato cultivar Diamant, but TEM shows that the inner wall is deeply lobed in some regions; these lobes could be interpreted as developing short wall ingrowths. TEM is also needed to confirm the presence or absence of abundant pit fields as reported for SYN of *Atalodera* (Mundo-Ocampo & Baldwin, 1983*a*). We hypothesize that modifications of the wall to enhance transport, including wall ingrowths and increased pit fields, may not be necessary in *Ekphymatodera* and perhaps other nematodes with relatively low energy demands on the host.

Host responses include complex characters which must be carefully understood to be accurately coded for phylogenetic analysis. Whereas the basic host response of a SUGC or SYN is not determined by the host, additionnal observations are needed to determine to what extent more subtle features are affected by the host or environment. Furthermore, characters of host response can only be considered for phylogenetic analysis with a large matrix of additional reliable features. A large matrix will strenghen, for example, parsimony approaches to identifying cases of parallel evolution of the SYN within Heteroderinae. Ultimately, features of host response, including the SUGC and SYN, may be shown to correspond to particular components of nematode stylet exudate. Such components of the exudate may prove to be less ambiguous characters for phylogenetic coding than characters based on their effects as expressed in the host.

References

ACEDO, J. R., DROPKIN, V. H. & LUEDDERS, V. D. (1984). Nematode population attrition and histopathology of *Heterodera glycines - soybean* associations. *J. Nematol.*, 16 : 48-57.

BALDWIN, J. G. & BELL, A. H. (1985). *Cactodera eremica* n. sp., *Afenestrata africana* (Luc *et al.*, 1973) n. gen., n. comb., and emended diagnosis of *Sarisodera* Wouts and Sher, 1971 (Heteroderidae). *J. Nematol.*, 17 : 187-201.

BALDWIN, J. G. & SCHOUEST, L. P. (1990). Comparative detailed morphology of the Heteroderinae Filip'ev & Shuurmans Stekhoven, 1941, *sensu* Luc *et al.* (1988) : phylogenetic systematics and revised classification. *Syst. Parasitol.*, 15 : 81-106.

BURROWS, P. R. (1990). Initiation and development of the syncytial feeding sites of cyst nematodes : A molecular view. *Nematologica*, 36 : 337 [Abstr.].

COHN, E., KAPLAN, D. T. & ESSER, R. P. (1984). Observation on the mode of parasitism and histopathology of *Meloidodera floridensis* and *Verutus volvingentis* (Heteroderidae). *J. Nematol.*, 16 : 256-264.

ENDO, B. Y. (1964). Penetration and development of *Heterodera glycines* in soybean roots and related anatomical changes. *Phytopathology*, 54 : 79-88.

FELDMESSER, J. (1953). *The life cycle of the golden nematode* Heterodera rostochiensis *Wollenweber, 1923, on tomato and a cytological study of its effects on the host.* Doctoral Dissertation. New York University, 55 p.

JOHNSON, P. W. & FUSHTEY, S. F. (1966). The biology of the oat cyst nematode *Heterodera avenae* in Canada. II. Nematode development and related anatomical changes in roots of oats and corn. *Nematologica*, 12 : 630-636.

JONES, M. G. K. (1981). Host cell responses to endoparasitic nematode attack : structure and function of giant cells and syncytia. *Ann. appl. Biol.*, 97 : 353-372.

JONES, M. G. K. & DROPKIN, V. H. (1975). Cellular alterations induced in soybean roots by three endoparasitic nematodes. *Physiol. Plant Path.*, 5 : 119-124.

JONES, M. G. K. & NORTHCOTE, D. H. (1972). Nematode-induced syncytium - A multinucleate transfer cell. *J. Cell Sci.*, 10 : 789-809.

KARNOVSKY, M. J. (1965). A formaldehyde-glutaraldehyde fixation of high osmolarity for use in electron microscopy. *J. Cell Biol.*, 27 : 137 [Abstr.]

KIM, Y. H. KIM, K. S. & RIGGS, R. D. (1986). Morphological characteristics of syncytia in susceptible hosts infected by soybean cyst nematode. *Phytopathology*, 76 : 913-917.

MELILLO, M. T., BLEVE-ZACHEO, T. & ZACHEO, G. (1990). Ultrastructural response of potato roots susceptible to cyst nematode *Globodera pallida* pathotype Pa 3. *Revue Nématol.*, 13 : 17-28.

MUNDO-OCAMPO, M. & BALDWIN, J. G. (1983*a*). Host parasite relationship of *Atalodera* spp. (Heteroderidae). *J. Nematol.*, 15 : 234-243.

MUNDO-OCAMPO, M. & BALDWIN, J. G. (1983*b*). Host response to *Sarisodera hydrophila* Wouts & Sher, 1971. *J. Nematol.*, 15 : 259-268.

MUNDO-OCAMPO, M. & BALDWIN, J. G. (1983*c*). Host response to *Meloidodera* spp. (Heteroderidae). *J. Nematol.*, 15 : 544-554.

MUNDO-OCAMPO, M. & BALDWIN, J. G. (1984). Comparison of host response of *Cryphodera utahensis* with other Heteroderidae and a discussion of phylogeny. *Proc. helminth. Soc. Wash.*, 5 : 25-31.

MUNDO-OCAMPO, M. & BALDWIN, J. G. (1991). Comparison of host parasite relations of *Ekphymatodera thomasoni* with other Heteroderidae. *Nematologica*, 36 (1990) : 375 [Abstr.].

SUAREZ, Z., SOSA-MOSS, C. & INSERRA, R. N. (1985). Anatomical changes induced by *Punctodera chalcoensis* in corn roots. *J. Nematol.*, 17 : 242-244.

VENABLE, J. H. & COGGESHALL, R. E. (1965). A simplified lead citrate stain for electron microscopy. *J. Cell Biol.*, 25 : 407-408.

VOVLAS, N. (1985). Morphology and histopathology of the cereal cyst nematode *Heterodera avenae* Woll. attacking wheat, oats and barley in Italy. *Nematol. medit.*, 13 : 87-96.