Host-Parasite Relationships of Atalodera spp. (Heteroderidae)

M. MUNDO-OCAMPO and J. G. BALDWIN[1]

Abstract: Atalodera ucri, Wouts and Sher, 1971, and *A. lonicerae,* (Wouts, 1973) Luc *et al.,* 1978, induce similar multinucleate syncytia in roots of golden bush and honeysuckle, respectively. The syncytium is initiated in the cortex; as it expands, it includes several partially delimited syncytial units and distorts vascular tissue. Outer walls of the syncytium are relatively smooth and thickest near the feeding site of the nematode; inner walls are interrupted by perforations which enlarge as syncytial units increase in size. The cytoplasm of the syncytium is granular and includes numerous plastids, mitochondria, vacuoles, Golgi, and a complex network of membranes. Nuclei are greatly enlarged and amoeboid in shape. Although more than one nucleus sometimes occur in a given syncytial unit, no mitotic activity was observed. Syncytia induced by species of *Atalodera* chiefly differ from those of *Heterodera sensu lato* by the absence of cell wall ingrowths; wall ingrowths increase solute transport and characterize transfer cells. In syncytia of *Atalodera* spp., a high incidence of pits and pit fields in walls adjacent to vascular elements suggests that in this case plasmodesmata provide the pathway for increased entry of solutes. The formation of a syncytium by species of *Atalodera* and *Heterodera sensu lato,* but a single uninucleate giant cell by *Sarisodera* and *Hylonema,* indicates a pattern of host responses that may be useful, with other characters, for phylogenetic inference for Heteroderidae. *Key words:* Heteroderoidea, histopathology, syncytium, giant cell.

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Nematode species of Heteroderoidea are characterized by complex host-parasite relationships in which they induce the host tissue to form specialized cells to sustain the nematode's sedentary feeding parasitic stages (23). Cells induced may be included in three categories as previously defined (3,23): 1) syncytia (e.g., *Heterodera* spp. *sensu lato*), 2) coenocyte (e.g., *Meloidogyne* spp.), 3) single uninucleate giant cell (SUGC; e.g., *Hylonema ivorense* and *Sarisodera hydrophila*).

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The basic type of host response is apparently a function of the nematode rather than the host, since in a given host, nematodes of different genera induce a response typical to the nematode genus (13,18,23,26) and the basic type of host response for a given genus (e.g., *Heterodera* spp., *Meloidogyne* spp.) is consistent among a large variety of host species (2,3,5,6,7,8,11,13,14, 21,27).

Recently, we reported that *Sarisodera hydrophila* Wouts and Sher, 1971 induces formation of a single uninucleate giant cell (SUGC) on each of three hosts examined (23). Although the host response differed from the syncytium of "closely related" *Heterodera sensu lato,* it did not necessarily

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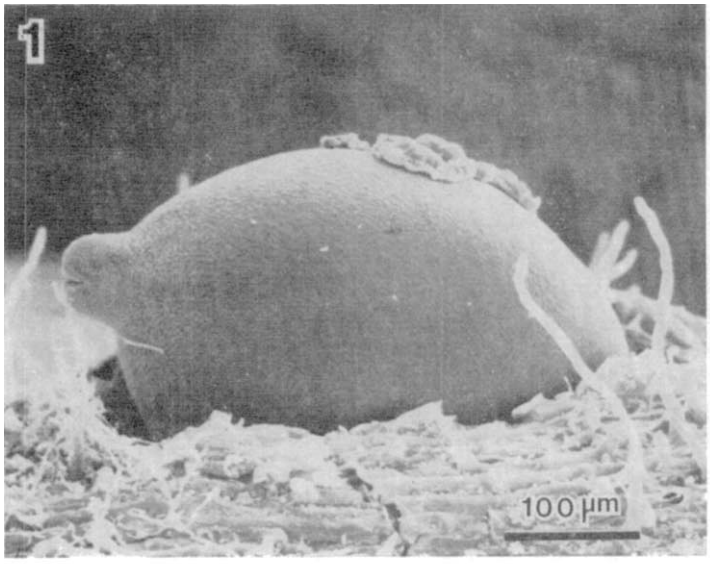
Figs. 1–7. SEM of *Atalodera* spp. and infected roots. 1) Mature female of *Atalodera ucri* embedded in host root. 2) Juveniles of *Atalodera lonicerae* embedded in host root. 3) Cross section showing hypetrophied cortical cells (C) surrounding a young female of *Atalodera ucri* (Ne). E = endodermis, VC = vascular cylinder. 4) Longitudinal section showing a syncytium (S) induced by *Atalodera ucri.* Co = columns, X = xylem. 5) Syncytial unit induced by *Atalodera lonicerae* showing columns (Co) and pit fields (PF) of cell wall. Cytoplasm removed by digestion. 6) Cell wall of a syncytium induced by *Atalodera ucri* adjacent to xylem elements with pits (P). Cytoplasm removed by digestion. 7) Cell wall perforations (Pe) between syncytial units of *Atalodera ucri.* Cytoplasm removed by digestion.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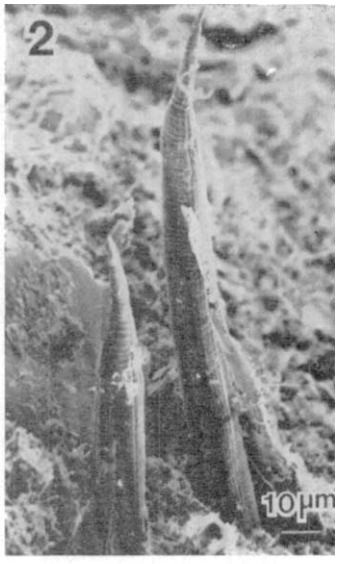
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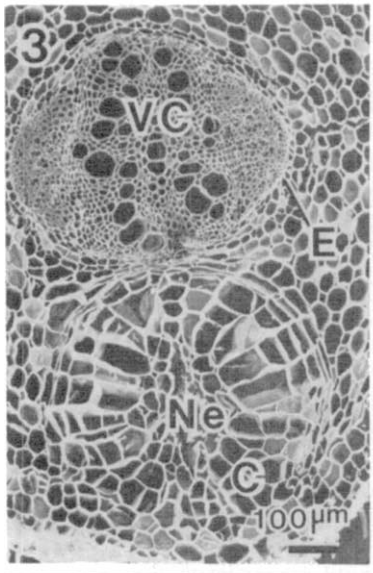
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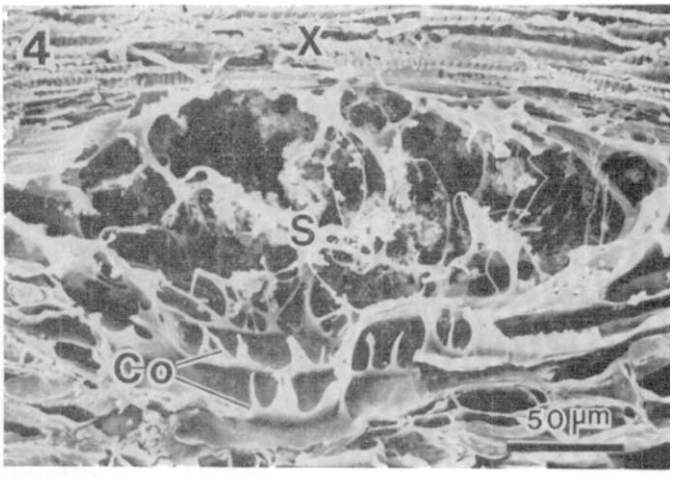
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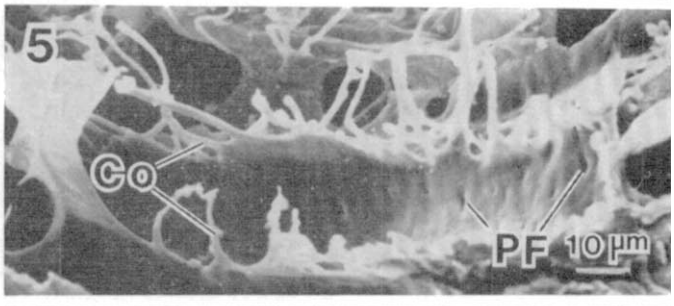
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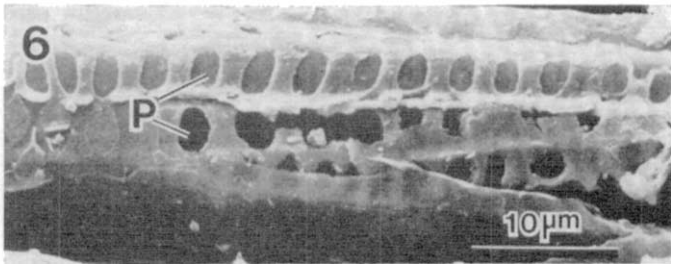
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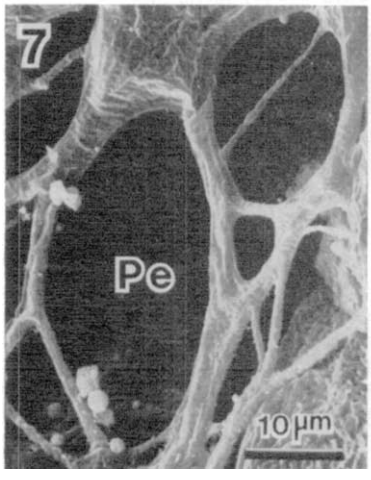
7
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indicate incongruency with existing hypotheses of phylogeny (9,23). Characterization of host responses of additional genera of Heteroderidae will be needed to determine whether concordance occurs between patterns of host responses of Heteroderidae in general and proposed phylogenies.

The present study characterizes host responses to *Atalodera ucri,* Wouts and Sher, 1971, and *Atalodera lonicerae,* (Wouts, 1973) Luc *et al.,* 1978. Both species were described on plants adapted to the semiarid climate of Southern California (28,29) and have a life cycle which is regulated by environmental conditions; development is seasonal from March to May and follows the typically rainy period.

MATERIALS AND METHODS

Atalodera ucri and *A. lonicerae* were collected on roots of the type hosts *Haplopappus palmeri* Gray (golden bush) and *Lonicera involucrata* (Richards) Banks ex Spreng (honeysuckle), respectively, at each type locality (28,29) during May 1981 and 1982. In addition, root samples infected with *A. lonicerae* were collected every week in March and every two weeks in April 1982 to observe the sequence of penetration of juveniles and development of the feeding site.

Root pieces were processed for histological examination, including bright field and Nomarski interference light microscopy (LM) as well as scanning (SEM) and transmission (TEM) electron microscopy, generally as previously described (23). Samples for bright field LM were fixed in formaldehyde, embedded in Paraplast-Plus, sectioned, and stained with safranin and fast green. Additional samples were fixed for Nomarski interference LM in glutaraldehyde, embedded in Spurr's resin, and sectioned with glass knives on an ultramicrotome. In some cases, sections were stained with toluidine blue (23).

Roots pieces were fixed for SEM in glutaraldehyde and were split to expose feeding sites of the nematodes. Some segments were treated by cytoplasm digestion technique (23). Samples were critical point dried, sputter coated with gold palladium, and examined with a JEOL JSM-35C scanning electron microscope at 15 KV (23).

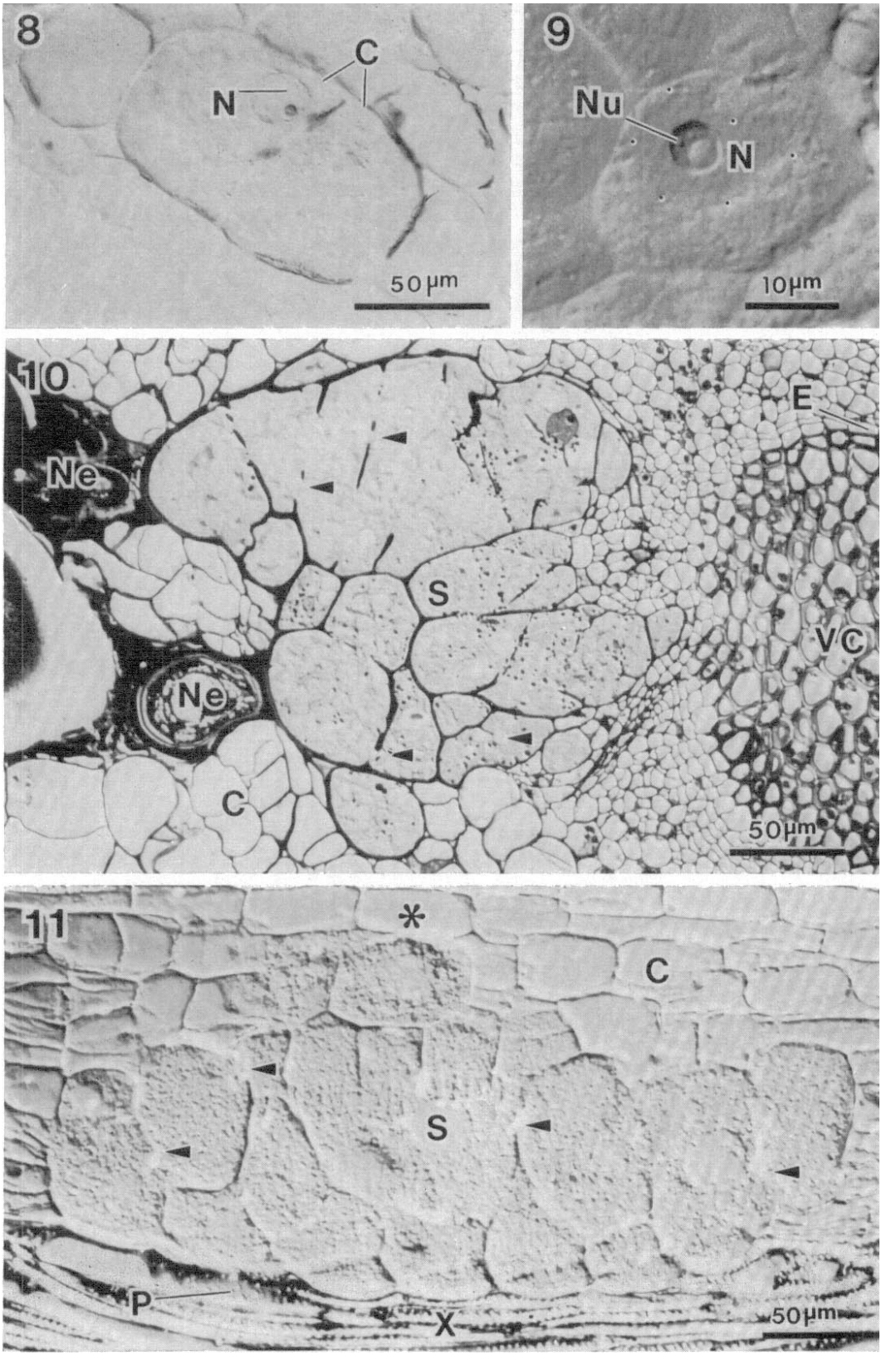
Root pieces were prepared for TEM by fixing in glutaraldehyde followed by postfixing in osmium tetroxide (OsO_4) and embedding in Spurr's resin. Sections were mounted on fomvar-coated grids, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-600 transmission electron microscope. (23).

RESULTS

Atalodera ucri and *A. lonicerae* induce similar multinucleate syncytia in roots of their respective type hosts, golden bush and honeysuckle. Surface observations indicated that nematodes were partially embedded in the newly developed roots (Fig. 1). No other symptoms such as galling or swelling of roots were observed. However, mature females occasionally were associated with external necrosis.

Juveniles of *Atalodera* spp. penetrate roots in early spring when plants show new growth. They most frequently initiate a feeding site on cortical tissue or endodermis. Feeding juveniles may be either partially or completely embedded in the cortex (Figs. 2, 3, 12). Cortical cells which contact the nematode tend to hypertrophy and often become necrotic (Fig. 3). In some cases necrosis was apparently exacerbated by secondary invaders, including fungi. The site where the nematode begins feeding is initially characterized by slightly enlarged cortical cells, which include granular cyto-

Figs. 8–11. LM (Nomarski) of sections of roots infected with *Atalodera* spp. 8) Hypertrophied cortical cells (C) showing initial phase of syncytial development induced by *Atalodera ucri,* including dense cytoplasm and enlarged nucleus (N). 9) Enlarged nucleus of a syncytium induced by *Atalodera ucri,* showing amoeboid shape. Dots (•) indicate boundary of nucleus. Nu = nucleolus. 10) Cross section of syncytium (S) adjacent to cortex (C), anterior end of two *Atalodera lonicerae* females (Ne), and vascular cylinder (VC). Arrowheads indicate cell wall perforations. E= endodermis. 11) Longitudinal section of syncytium induced by *Atalodera ucri* adjacent to xylem elements (X) with pits (P). Asterisk (*) indicates feeding position of nematode. C = cortex.


8
C
N
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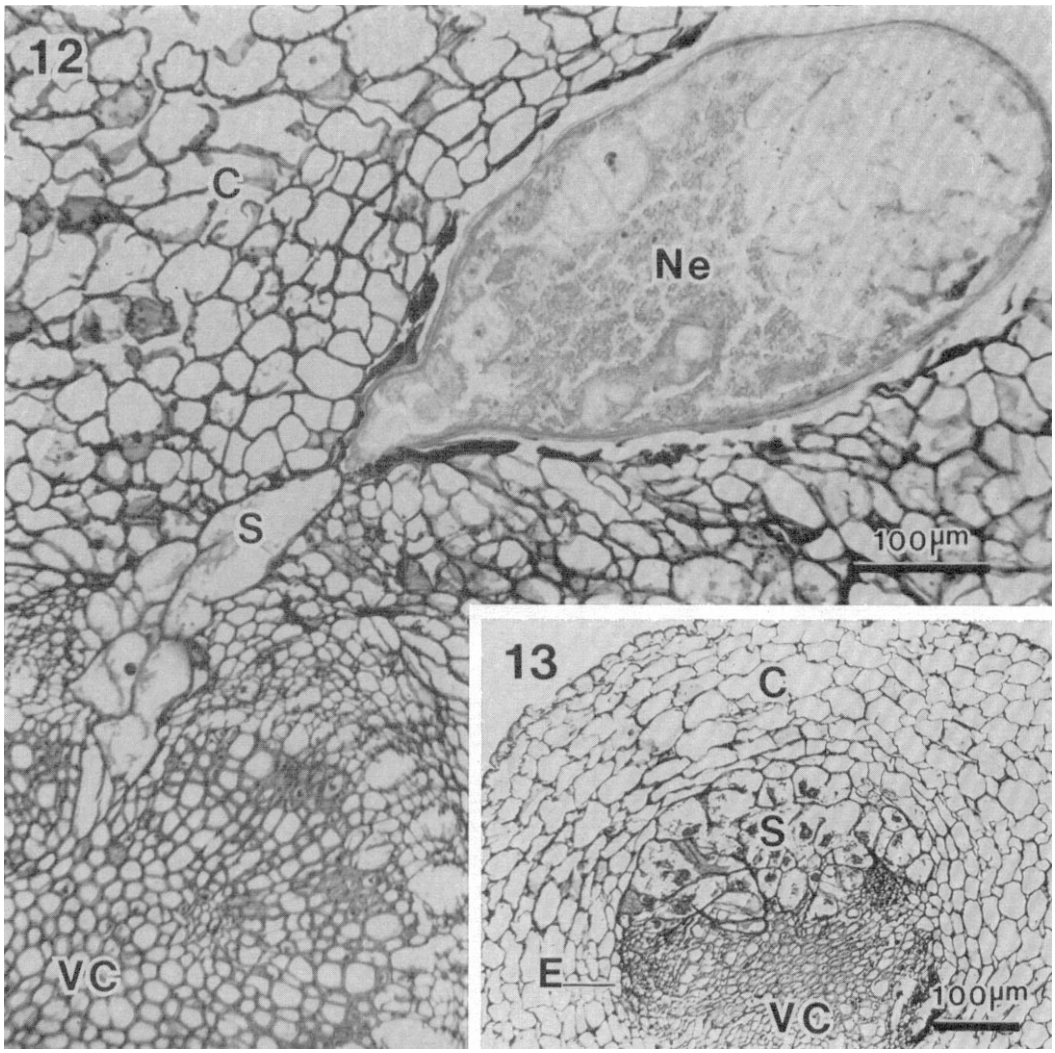
9
Nu
N
10 µm
10
Ne
E
S
VC
Ne
C
50 µm
11
*
C
S
P
X
50 µm

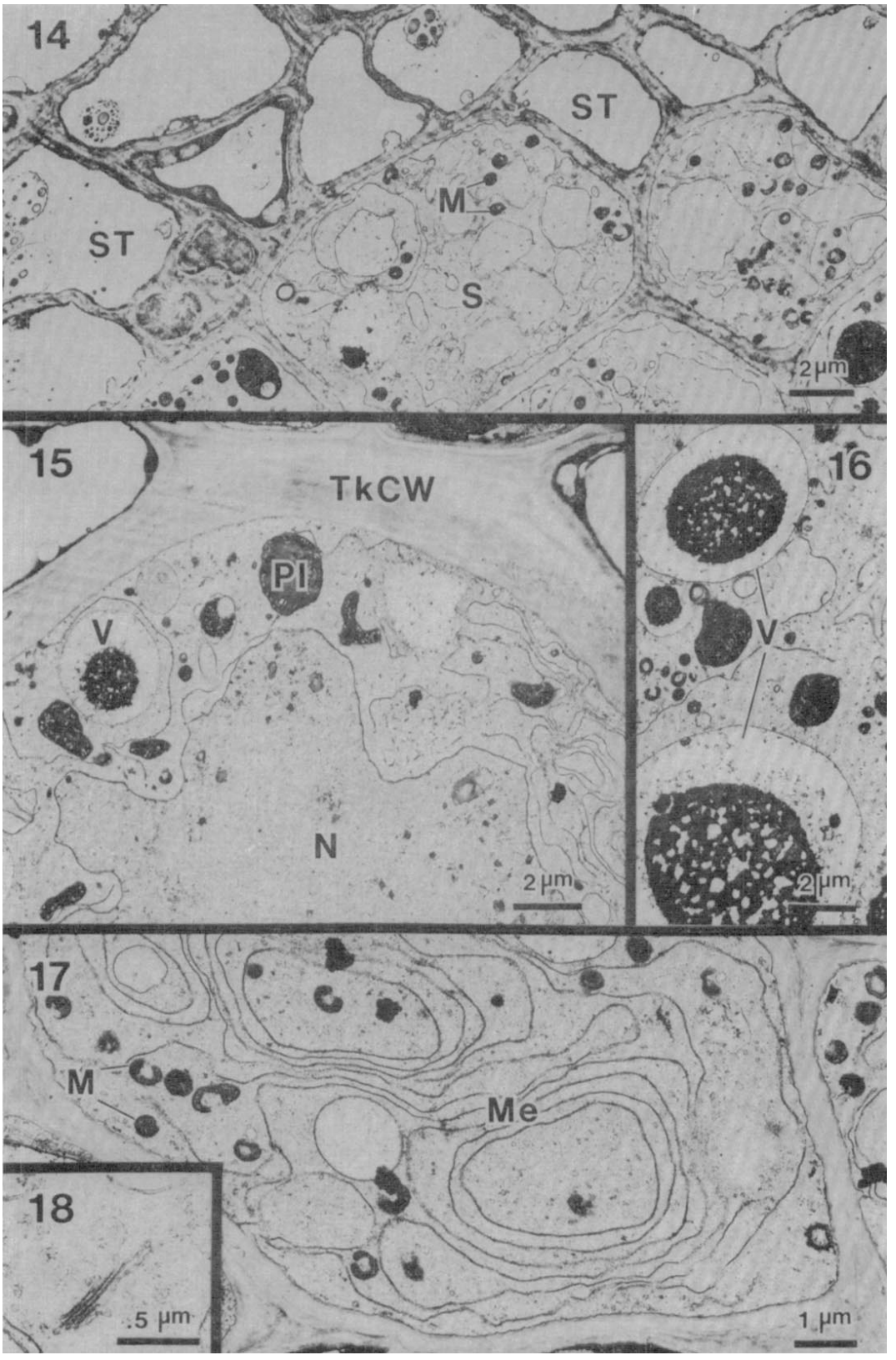
plasm and hypertrophied nuclei (Figs. 8, 9). Subsequently, as the nematode continues to feed and mature, the syncytial units ("cells" or chambers which together form the syncytium) increase in size. Eventually the syncytium reaches the vascular cylinder where it extends longitudinally in both directions relative to the axis of the root (Figs. 11, 12, 13). Syncytial units become less well defined and the outer boundary of the syncytium becomes better delineated from surrounding distorted tissues (Figs. 10, 12, 13).

A fully developed syncytium is irregular in shape and varies from 150 to 600 μm wide and from 400 to 800 μm long; shape and size vary with host, developmental stage of the roots, and nematode species.



Figs. 12–13. LM (bright field) of root cross sections. 12) *Atalodera lonicerae* female (Ne) in feeding position adjacent to syncytium (S). C = cortex, VC = vascular cylinder. 13) Mature syncytium induced by *Atalodera ucri* which distorts vascular cylinder (VC). C = cortex, E = endodermis.

Figs. 14-18. TEM of syncytia induced by *Atalodera lonicerae*. 14) Sieve tube elements (ST) adjacent to small peripheral syncytial units (S). Note absence of cell wall ingrowths. M = mitochondria. 15) Section showing thickened cell wall (TkCW) adjacent to anterior end of nematode. N = nucleus, Pl = plastids, V = vacuole. 16) Section showing vacuoles (V) containing dense bodies. 17) Section showing elaborate network of continuous membranes (Me). M = mitochondria. 18) Section showing Golgi.


14
ST
ST
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S
2 µm
15
TkCW
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2 µm
16
V
2 µm
17
M
Me
1 µm
18
.5 µm

Syncytia induced by *A. ucri* on golden bush tend to be larger than those induced by *A. lonicerae* on honeysuckle. A given syncytium is usually associated with a single nematode, but in rare cases more than one nematode may occur adjacent to a large syncytium (Fig. 10).

The outer walls which delimit mature syncytia are generally smooth and less than 0.5 μm thick, but they tend to be thicker (up to 3.0 μm) near the feeding site of the nematode (Figs. 10, 15). Cell walls of collapsed adjoining cells often become attached to syncytial walls resulting in a thick lamellae of wall material (Fig. 20). The inner walls shared between syncytial units are interrupted by perforations (Figs. 7, 10, 11, 19). As syncytial units enlarge and these perforations widen, wall fragments are reduced to columns (Figs. 4, 5). Cell wall protuberances were not detected on syncytial walls adjacent to vascular elements (Figs. 11, 13, 14). Instead, perforations, pits, and pit fields connecting vascular elements with syncytia were observed (Figs. 5, 6).

Nuclei and nucleoli of syncytia are larger than in normal cells and the nuclei are amoeboid in shape (Figs. 9, 15, 19). In serial sections of a given syncytium, frequently more than one nucleus is present per syncytial unit; however, mitotic activity was not observed.

The granular electron-dense cytoplasm of syncytia contains numerous dense plastids, irregularly-shaped mitochondria, Golgi, vacuoles, and a complex network of membranes (Figs. 15–20). Vacuoles are highly variable in size, frequently contain dense bodies, and tend to be closely associated with inner walls (Figs. 15, 16). The membranes occur as parallel arrays in syncytia induced by *A. ucri* (Fig. 19) and are continuous in syncytia induced by *A. lonicerae* (Fig. 17).

As females of *Atalodera* spp. become senescent, the cytoplasm of the associated syncytia becomes highly vacuolated and organelles disintegrate.

DISCUSSION

Atalodera ucri and *A. lonicerae* induce the same type of multinucleate syncytium in their respective hosts, golden bush and honeysuckle. Their syncytia share certain features with those induced by *Heterodera sensu lato* (22) and the nonheteroderids, *Rotylenchulus reniformis,* Linford and Oliveira, 1940, and *Nacobbus* spp. In each case, the response is initiated by enlarged cells with enlarged nuclei and nucleoli; next the cytoplasm becomes electron dense and syncytial units expand. Reportedly, as the result of dissolution of cell wall material, cell walls become discontinuous, providing a continuous cytoplasm for the nuclei (13,14).

Similarly to the syncytia of other heteroderids, the outer walls of syncytia of *Atalodera* spp. are thicker than those of adjacent cells (8,18,21). The extent of this thickening is variable, but typically it is greatest toward the feeding site of the nematode and decreases away from it. Perforations of inner walls (shared between syncytial units) vary in size and number. They are less abundant and smaller in the smaller syncytial units distant from the feeding site than in larger units adjacent to the nematode. This suggests that the syncytium is formed by a process of incorporation of neighboring cells, starting at the feeding site and continuing along the periphery furthest away from the nematode (1,8,10, 15).

Syncytia induced by *Heterodera* spp. *sensu lato,* and the single uninucleate giant cells (SUGC) associated with *Rotylenchulus macrodoratus* Dasgupta et al. 1967, are characterized by wall ingrowths or protuberances which when stained with toluidine blue, are easily resolved with LM and are also visible by SEM and TEM (4,13,14,15, 16,17,19). These ingrowths provide increased surface area for entry of solutes and are typical of transfer cells (24). Similar wall ingrowths occur in the coenocyte (multinucleate giant cell) induced by *Meloi-*

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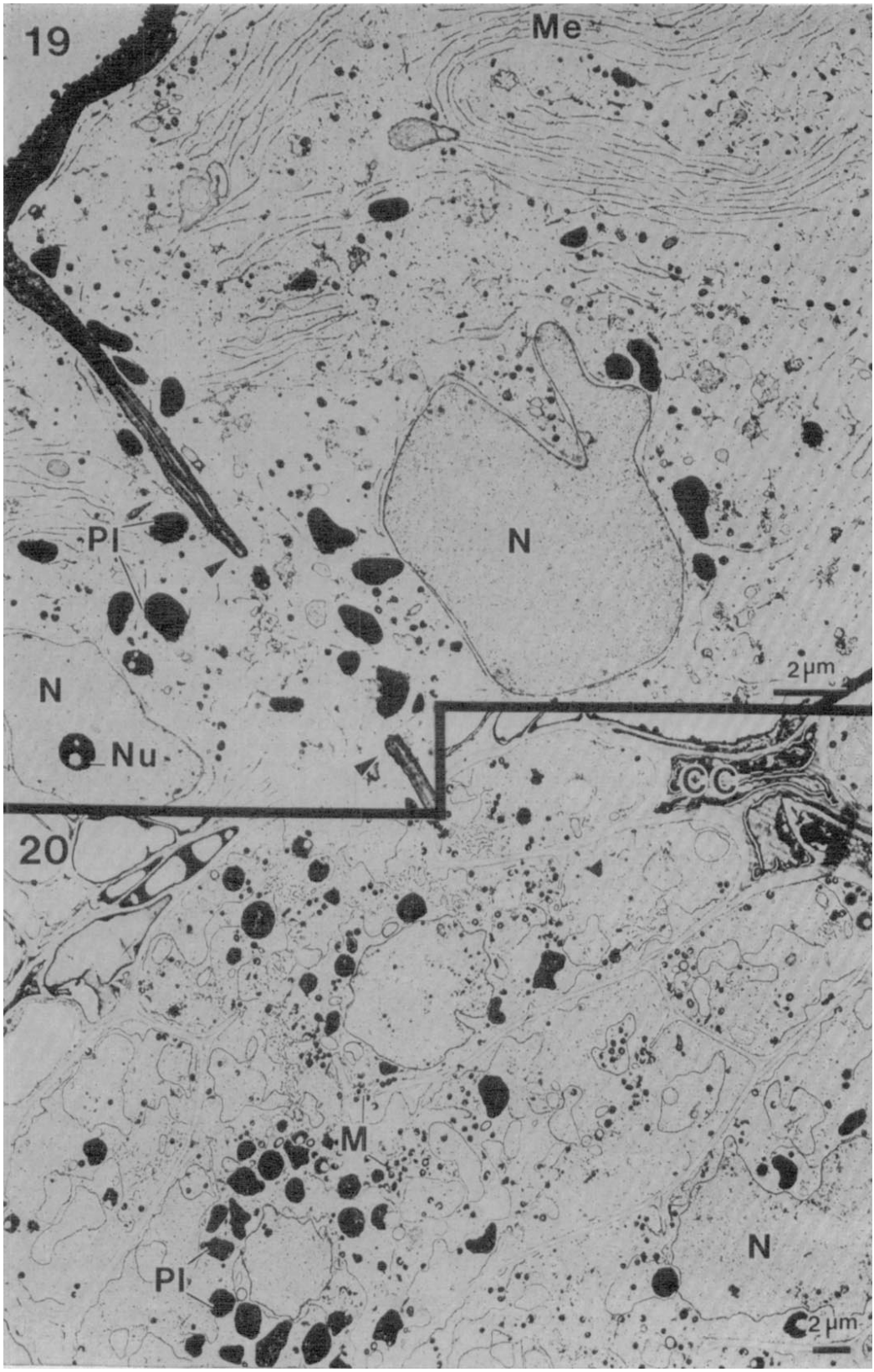
Figs. 19–20. TEM of syncytia induced by *Atalodera* spp. 19) Syncytium induced by *Atalodera ucri.* Arrowheads indicate limits of cell wall perforations. Me = membranes, N = nucleus, Nu = nucleolus, Pl = plastids. 20) Mature syncytium induced by *Atlodera lonicerae,* showing collapsed cells (CC) and organelles. M = mitochondria, N = nucleus, Pl = plastids.


19
Me
Pl
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2μm
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20
M
Pl
N
2μm

dogyne spp. In syncytia induced by *Atalodera* spp., *R. reniformis* (25), *Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944 (20), and in the uninucleate giant cell of *S. hydrophila* (23), no cell wall ingrowths were observed with LM, TEM, or SEM. Similarly, wall ingrowths were not observed in the uninucleate giant cell of *Hylonema ivorense* Luc et al. 1978 with LM (26). Jones (13) noted that absence of wall ingrowths correlates with a high frequency of plasmodesmata. In *Atalodera,* as in *R. reniformis, N. aberrans,* and *S. hydrophila,* plasmodesmata may provide the main pathway for entry of solutes (11,12,13,18,20,25). This particularly seems to be the case for *Atalodera,* where a large number of pits and pit fields with plasmodesmata interconnect syncytia and vascular elements.

The granular cytoplasm, organelles, and structures in syncytia induced by *Atalodera* spp. are similar to those in syncytia of other nematode species on a variety of hosts and generally indicate a high rate of metabolism (12). Vacuoles containing dense material are concentrated near inner walls, which suggests that they may be involved in cell wall dissolution. Histochemical studies would be useful in identifying the contents of these vacuoles and may elucidate their function. Elaborate networks of membranes within syncytia may reflect a high rate of metabolism. Differences observed between membranes of *A. ucri* and *A. lonicerae* could be due to the different hosts involved.

The different genera of Heteroderidae induce different host responses. *Sarisodera* and *Hylonema* induce SUGC, whereas *Atalodera* and *Heterodera sensu lato* induce a syncytium. Since the basic host response is determined by the nematode (i.e., not altered among hosts), it may be useful to test the pattern of host responses for concordance with hypotheses of phylogeny (23). The cladogram proposed by Ferris indicates that *Atalodera* shares a common ancestor with a large group including *Hylonema, Sarisodera,* and *Heterodera sensu lato* (9). If this proposal is correct and if the ancestor was associated with a SUGC, the capacity to induce a syncytium necessarily would have arisen at least twice, once in *Atalodera* and once in *Heterodera sensu lato.* Similarly, if the ancestor induced a syncytium, the capacity to induce SUGC would have arisen at least twice. Basic differences between the syncytium of *Atalodera* and *Heterodera sensu lato,* such as the absence of wall ingrowths in *Atalodera,* might indicate that the formation of syncytia is the result of parallel evolution. The significance of host responses as a character to test hypotheses developed from classical data will be better understood as new characters and character states are identified and host responses of additional species are described.

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