An unique host-parasite relationship between *Hylonema ivorense* (Nematoda : Heteroderidae) and the roots of a tropical rainforest tree [1]

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SUMMARY

Roots of young avodire *(Turraeanthus africana)*, a member of the Meliaceae used as a source of lumber, were observed in Ivory Coast to have slight swellings frequently with a small necrotic depression. Dissection of these swellings revealed the presence of a necrotic tunnel leading from the necrotic depression to a saccate female of *Hylonema ivorense* usually located near the vascular system. Histological examination of infected roots showed that each female incited production of only one giant cell, each giant cell was uninucleate, each nucleus was greatly enlarged-up to 200 times the volume of a normal nucleus, and each nucleus contained an enlarged nucleolus itself usually larger than a healthy nucleus. No discrete cell wall was observed surrounding the giant cell. Xylem tissue near infection sites was abnormal. Eggs, and occasionally juveniles, were found throughout the tunnel. Although a suitable host-parasite relation exists, infected tissue is characterized by considerable necrosis. Giant cell formation and nuclear enlargement were detected in ten-day-old material. Enlargement of these structures continued through 40 days. Two additional hosts, *Neuropeltis prevosteoides* and *Khaya ivorensis,* exhibited the same symptoms. *Tabernaemontana crassa* was also observed to be naturally infected.

RÉSUMÉ

Un mode unique de relations parasitaires chez Hylonema ivorense *(Nematoda : Heteroderidae) attaquant les racines d'un arbre de la forêt tropicale humide.*

Les racines de jeunes avodirés (*Turraeanthus africana,* Meliacées), essence exploitée en Côte d'Ivoire pour l'ébénisterie, montraient de légers renflements souvent accompagnés en surface d'une dépression nécrotique. A la dissection, ces renflements ont révélé la présence d'un tunnel nécrotique conduisant de la dépression à une femelle renflée de *Hylonema ivorense,* généralement localisée près du cylindre central. L'étude histologique des racines infestées a montré que chaque femelle induit la formation d'une cellule géante unique, possédant un seul noyau anormalement développé (plus de 200 fois le volume d'un noyau normal) contenant lui-même un nucléole hypertrophié, d'une taille supérieure à celle d'un noyau normal. Aucune paroi, même discrète, n'a été observée autour des cellules géantes. Les tissus du xylème sont perturbés autour des sites d'infestation. Des œufs et occasionnellement des juvéniles ont été observés à l'intérieur du tunnel. Bien qu'un modede relation hôte/parasite efficace paraisse exister, les tissus infestés sont caractérisés par l'étendue considérable des zones nécrotiques. La formation des cellules géantes et l'hypertrophie de leur noyau ont pu être observées sur du matériel végétal n'ayant pas plus de dix jours, ces phénomènes se poursuivant pendant 40 jours. Deux autres plantes hôtes, *Neuropeltis prevosteoides* et *Khaya ivorensis,* présentent les mêmes symptômes. *Tabernaemontana crassa* est également infesté en milieu naturel.

Cellular responses of plants to plant-parasitic nematodes have been thoroughly reviewed (Dropkin, 1969), from which it is obvious that effects of parasitism vary greatly depending upon the species of nematode involved.

Members of the Heteroderidae (Filipjev & Schuurmans Stekhoven, 1941) Skarbilovich, 1947 and the Meloidogynidae (Skarbilovich, 1959) Wouts, 1973 induce the most profound anatomical changes in the host. Members of

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the genus *Meloidogyne* Goeldi, 1892 usually produce root galls characterized by hypertrophy and hyperplasia of various tissues and by the presence of large, multinucleate giant cells, or syncytia (Dropkin, 1969). Members of the genus *Heterodera* Schmidt, 1871 normally do not induce galls, but the histopathology of infection sites is similar to that of *Meloidogyne*. It is generally conceded that these syncytia, two to many per infection, are formed by fusion of cells at the feeding site resulting in the formation of abnormally enlarged, multinucleate cells (Rohde & McClure, 1975). Mitotic division, frequently reported to be synchronous, contributes to the multinucleate condition ; in certain *Meloidogyne* infections syncytia have been reported to have "hundreds of nuclei" (Dropkin, 1969). These nuclei are frequently enlarged, sometimes one hundred times normal (Krusberg & Nielsen, 1958 ; Dropkin & Nelson, 1960). Nucleoli are also frequently enlarged in *Meloidogyne* infections and have been reported to be as large as normal nuclei (Krusberg & Nielsen, 1958). The host-parasite relations of *Meloidodera floridensis* has been partially described by Ruehle (1962), who stated that "giant cells adjacent to the female nematode" are produced but no mention was made of their nuclear condition. The histopathology induced by other members of the Heteroderidae, i.e. *Cryphodera* Colbran, 1966, *Atalodera* Wouts & Sher, 1971, *Sarisodera* Wouts & Sher, 1971 and *Hylonema* Luc, Taylor & Cadet, 1978, has not been described.

The histopathology of roots of the tropical rainforest tree, avodiré, infected with *Hylonema ivorense* Luc, Taylor & Cadet, 1978, is unlike any known caused by a member of the Heteroderidae and is, therefore, described in detail.

Materials and Methods

Soil and root samples were collected from avodiré, *Turraeanthus africana* Pellegr., a member of the Meliaceae, growing in a tropical rainforest reserve at Banco near Abidjan, Ivory Coast. Nematodes were extracted by standard techniques. Small swellings, each usually with a small necrotic depression, were noted on the roots. Root pieces exhibiting swellings were cut and fixed in FAA for 72 hours or longer. They were then embedded in paraffin, sectioned at 15 or 18 μm and stained with safranin and fast green. Photographs were taken with a Leitz "Orthomat" photomicroscope. Additional fixed roots were dissected by hand to further study the relationship.

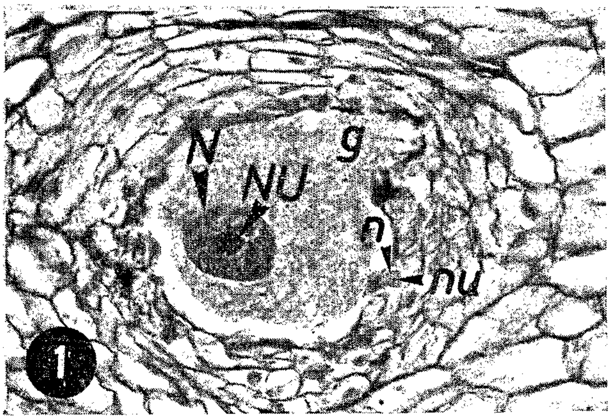
To study symptom development, seeds of *T. africana* were germinated and seedlings transplanted to sterile soil. Suspensions of *H. ivorense* juveniles were poured over the soil surface around the seedlings. One infected plant was sacrificed every five days and the infected roots were fixed, sectioned and stained as described above.

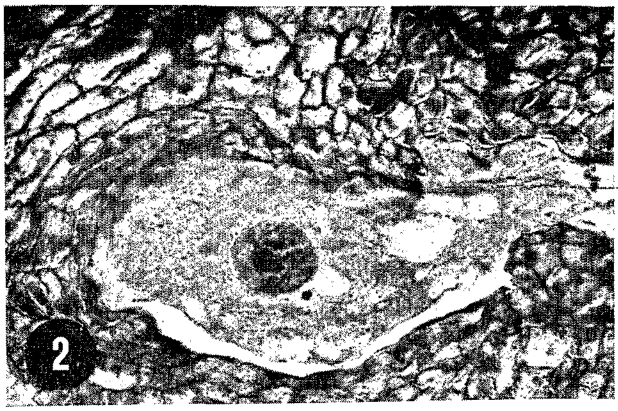
Results

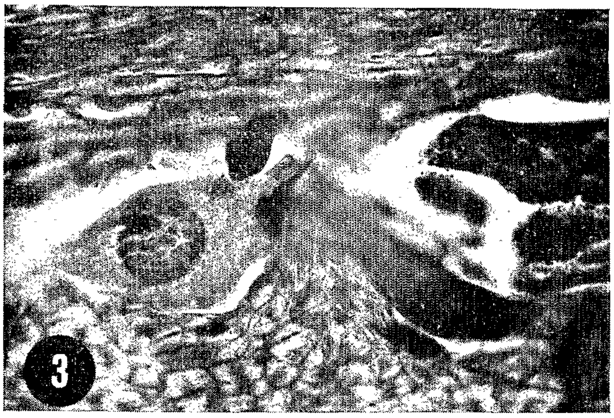
Observations on naturally infected roots

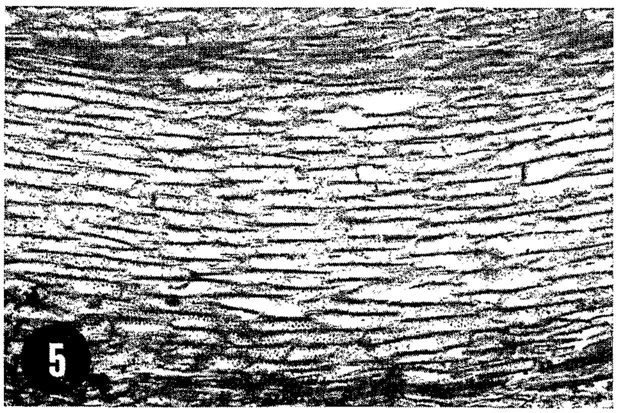
Nucleus and nucleolus. Figure 1 illustrates a giant cell induced by *H. ivorense* in a root of *T. africana*. The most conspicuous feature of this cell is the enlargement of the single nucleus (N), especially when compared to the size of the nucleus (n) in an adjacent unaffected cell. When 25 nuclei from similar giant cells were measured their mean diameters were 78 × 59 μm ; normal nuclei measured 11 × 10 μm. Calculation of the volume of the larger nuclei indicate that the mass of these nuclei can be more than 200 times that of normal nuclei. The single, deeply staining nucleolus (NU) is also greatly enlarged, 17 × 15.5 μm, compared to a diameter of less than 1.6 in healthy cells. In examining hundreds of giant cells, no case of multiple nuclei was observed.

Giant cells. Examples of *H. ivorense*-induced giant cells are shown in Figures 1-4. Giant cells were found in the cortex (Fig. 1), adjacent to the vascular tissue (= pericycle?) as in Figure 3, and within the vascular tissue (Figure 4 illustrates a giant cell surrounded by xylem tissue). These cells are irregular in outline, although in the xylem they are generally elongated in the direction of the root axis (Fig. 4). They are extremely variable in size and lengths of over 350 μm have been measured. In addition to









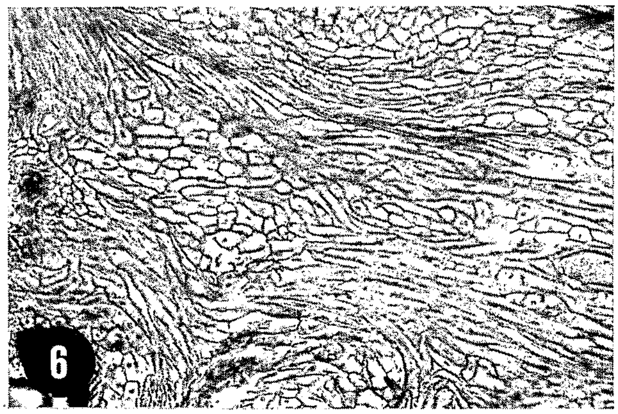


Fig. 1-4 : Giant cells induced by *Hylonema ivorense* in roots of *Turraeanthus africana* (g = giant cell ; N = enlarged nucleus ; NU = enlarged nucleolus ; n = normal nucleus ; nu = normal nucleolus).

Fig. 5-6 : Xylem tissue in roots of *T. africana ;* 5 : non-infected root portion ; 6 : root infected with *H. ivorense.*

being uninucleate, as previously mentioned, the giant cell is characterized by dense, deeply staining cytoplasm. No discrete cell wall has been observed surrounding giant cells (Fig. 1-3). The apparent "wall" in Figure 4 was, in fact, composed of the cell walls of adjacent xylem tissue. It must be emphasized that in no case was more than one giant cell associated with a single nematode. When more than one giant cell was observed in a root section, examination of adjacent serial sections always demonstrated the presence of multiple infections. Cortical cells adjacent to or near the giant cells frequently appeared smaller and more compact than cells in healthy tissue (Fig. 1 and 11).

Abnormal xylem. Xylem tissues in non-infected portions of the roots of *T. africana* have a regular appearance (Fig. 5), in contrast to heavily infected roots in which xylem is disoriented and cells are of atypical size and shape. Variability of this cellular configuration is shown in Figure 6.

Tunnel. Another characteristic of this host-parasite relationship is the presence of a "tunnel" extending from the posterior portion of the mature female to the root surface. In unsectioned material fixed in FAA, the tunnel appears as a reddish-purple tissue and upon dissection it usually remains attached to the female (Fig. 7). The tunnel is harder in texture than adjacent root tissue. In sectioned roots the tunnel stains deep red and appears to be composed of compact, dead cells and cellular debris. In areas of the tunnel wall where cellular integrity exists, cell walls are thickened and stain a deep red and the cells are devoid of protoplasm. In the central hollow portion of the tunnel, eggs and occasionally juveniles of *H. ivorense* are found (Fig. 8). Eggs occur along the total length of the tunnel and frequently a few are seen at the root surface in what appears as a small amount of gelatinous material. The entire aspect of this relationship is illustrated in Figure 9. Soil fungi readily colonize the tunnels as indicated by the frequent presence of mycelium amongst the cellular debris.

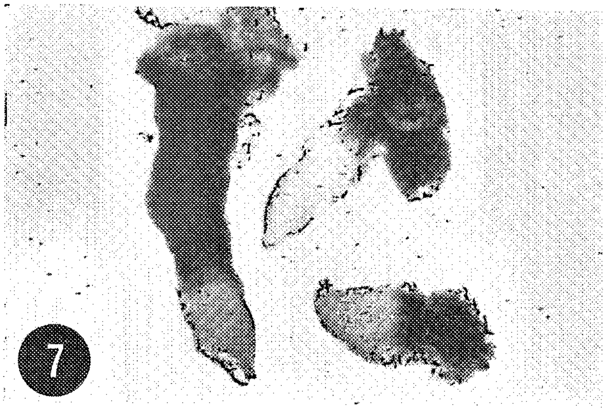
Relation of H. ivorense *to giant cells and other tissues.* The head of *H. ivorense* was frequently found closely appressed to the giant cell (Fig. 10 and 11) and sometimes apparently extending into the giant cell near the enlarged nucleus (Fig. 3). A cast juvenile cuticle can often be seen surrounding the lip region (Fig. 11, 12, 13 and 15). In many cases observed there appears to be another structure surrounding the anterior end of the nematode which, based upon staining reaction, is presumed to be of plant origin (Fig. 10 and 11). In Figure 12, the anterior end of the nematode (1) is obviously within an annulated juvenile cuticle (2) with a distinct lip region ; however, the other structure (3) has no distinct morphology to indicate if it is of nematode or plant origin. In Figure 13, the structure surrounding the nematode is clearly a cast cuticle with the anterior portion of the stylet (S) still attached. In contrast to that, Figure 14 shows the lip region of *H. ivorense* closely appressed to the giant cell with neither a cuticle nor any other structure present.

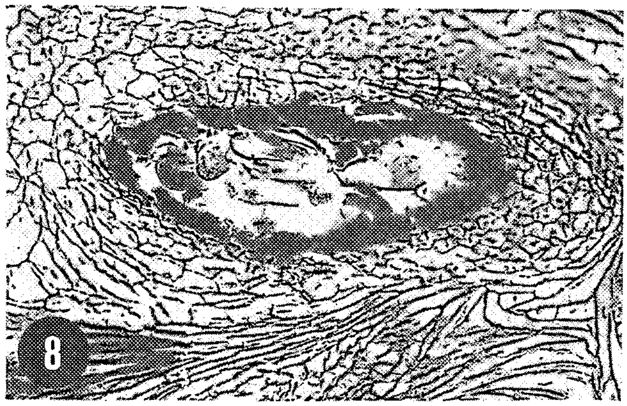
A common feature of this host-parasite relationship is the presence of necrotic plant tissue adjacent to the body of the nematode and often extending some distance beyond. In Figure 14, an infection site of a root inoculated 40 days before, an amorphous necrotic region occurs adjacent to the giant cell in which the nematode lip region is embedded. In Figure 17, a fifteen-day-old infection, a necrotic region extends to the left side of the photograph surrounding the anterior end of the nematode ; in fact, this necrotic vascular tissue extends along the entire length of the juvenile (Fig. 21) and beyond. It is suggested that such a necrotic zone ultimately develops into the tunnel, the necrotic nature of which has already been mentioned.

Infected roots usually possess small swellings with a slight depression in the center, corresponding to the tunnel opening. Sections through such swellings indicate an increase in number of cortical cells but no general increase in their size (Fig. 9). Much of the volume of the swelling is occupied by the tunnel.

Early stages of symptomatology

Penetration by juveniles of *H. ivorense* was observed in roots of *T. africana* five days after inoculation. Moderate (Fig. 22) to severe





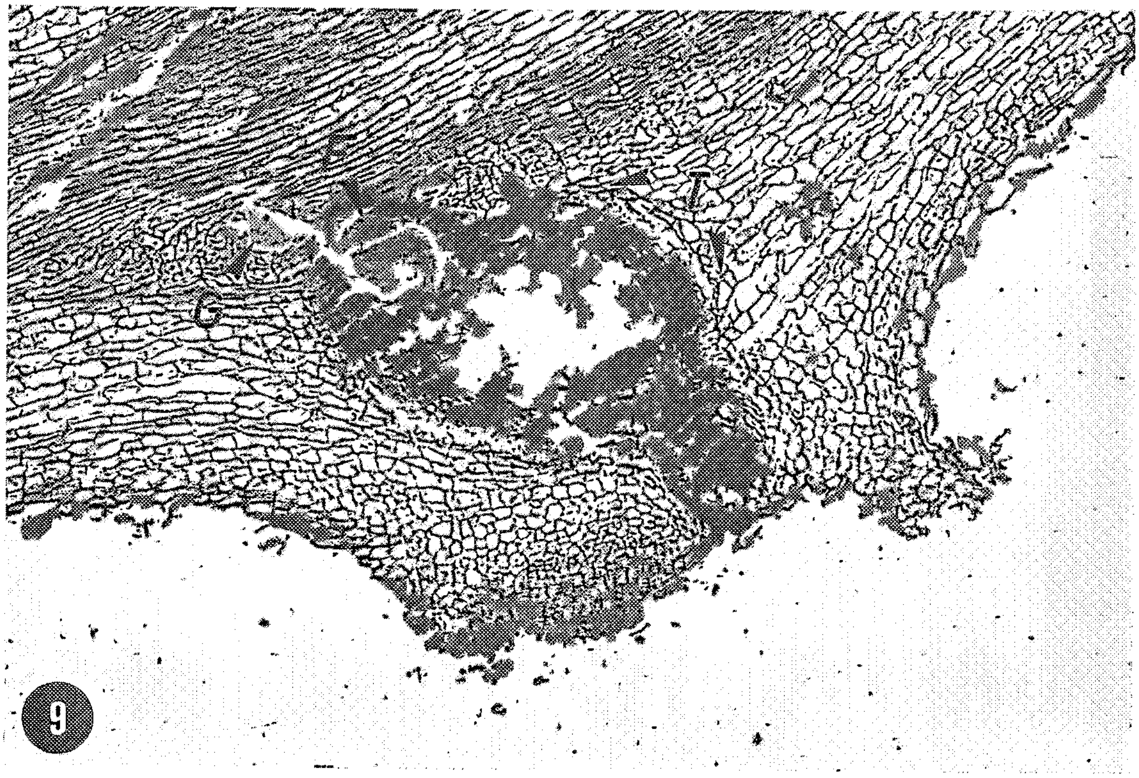


Fig. 7 : Three females of *Hylonema ivorense* (light) dissected from root showing attached tunnels (dark).

Fig. 8 : Cross-section through tunnel showing deeply stained wall and eggs in hollow portion. Note abnormal xylem.

Fig. 9 : Longitudinal section through infected root of *Turraeanthus africana* showing giant cell (G), nature female (F) and tunnel (T) extending to root surface.

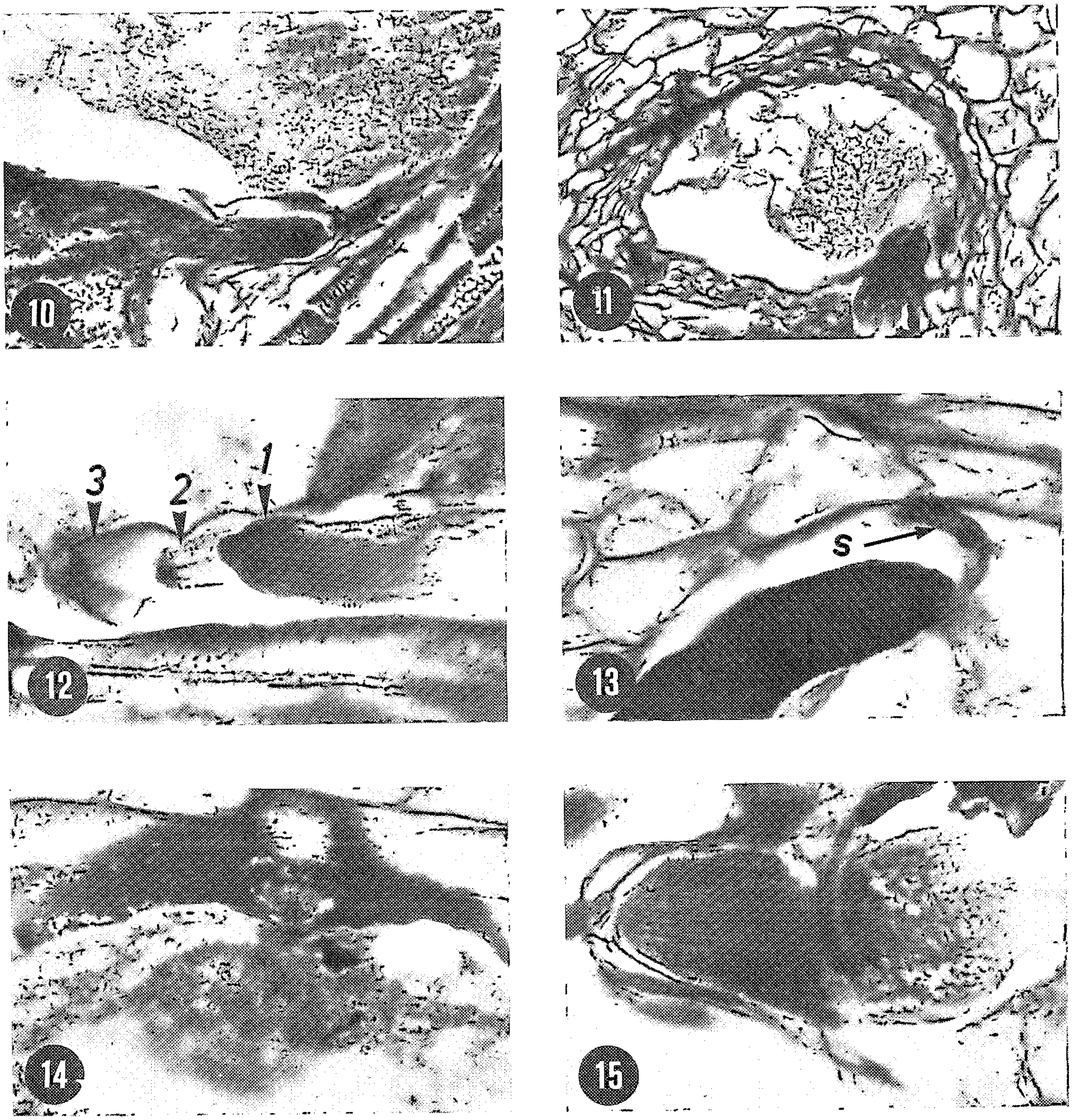


Fig. 10-15 : Relationship between anterior end of *Hylonema ivorense* and giant cells of *Turraeanthus africana*. Explanation of markings is given in the text.

(Fig. 23) necrosis, indicated with arrows, was present in tissues adjacent to the juveniles (J) ; however, no indication of giant cell formation could be detected.

Giant cell formation was first observed in ten-day-old material. One cell (Fig. 16) measured 108 × 29 μm with a nucleus 26 × 21 μm (normal nucleus = 11 μm) and a nucleolus 5.6 μm in diameter (normal nucleolus less than 1.6 μm). Necrosis near juveniles was conspicuous.

Figure 17 illustrates a typical giant cell fifteen days after inoculation. The cell is 160 × 75 μm with a nucleus 29 μm in diameter and a nucleolus 6.4 μm in diameter. The largest nucleus observed at this stage had a diameter of 32 μm.

After twenty days, further increase in size of giant cells, nuclei and nucleoli was observed. The nucleus in Figure 18 is 33 × 32 μm. Abnormal xylem was observed near infection sites for the first time in material of this age.

Figure 19 shows a giant cell in 25-day-old material. The nucleus measures 42 × 35 μm and the nucleolus 11 × 8 μm. Examination of another section through the same nematode shown in Figure 14 revealed the presence of a partially shed cuticle behind the head region — the earliest observation of molting of this nematode.

Between 25 and 40 days, development of nematodes and affected tissues continued. Figure 20 shows a giant cell 40 days after inoculation with a nucleus 51 × 35 μm having a nucleolus 9.6 μm in diameter. Another cell in the same root had a nucleus 61 × 48 μm. Many molting specimens were present in the 40-day-old material (Fig. 12 and 13).

No subsequent development could be observed in the older material available for study at this time.

Other hosts

Roots collected from various plants near the type locality were examined for *Hylonema ivorense.* Roots of three other species were found to be naturally infected with this nematode : *Neuropeltis prevosteoides* Mangenot (Convolvulaceae) ; *Tabernaemontana crassa* Benth. (Apocynaceae) ; and an unidentified tree. In addition, successful inoculations have been made on *Khaya ivorensis* Chev. (Meliaceae). Roots of *N. prevosteoides* and *K. ivorensis* were examined histologically and the same host-parasite relationships as described for avodiré were found (Fig. 24 and 25).

Discussion

On the basis of the observations described above, we consider that the combination of pathological effects of *Hylonema ivorense* on its hosts is unique among plant-parasitic nematodes. The combination of symptoms including only one giant cell per parasite, a single greatly hypertrophied nucleus containing a single hypertrophied nucleolus, associated abnormal xylem, and the necrotic tunnel containing eggs, has not been previously described.

The single uninucleate giant cell most closely resembles that induced in soybean roots by *Rotylenchulus macrodoratus* (Cohn & Mordechai, 1976 ; 1977). However, they reported that the nucleus was irregular in shape whereas that induced by *H. ivorense* is ovoid to spherical. They also reported that the nucleus occupies up to one-third of the cell area which is greater in relation to the size of the cell than is the enlarged nucleus of giant cells induced by *H. ivorense.* An uneven thickened wall surrounds the cell induced by *R. macrodoratus*, whereas no such wall has been demonstrated in our material.

The validity of using the term, "giant cell", to describe these structures is contrary to general usage, eg. Endo (1971, p. 92) stated that the term "giant cell" refers to a "multinuclear unit". Rather than propose a new term for an uninucleate hypertrophied cell or unit, we prefer to consider that the term "giant cell" includes any hypertrophied cell or unit induced in a host by a plant-parasitic nematode regardless of its nuclear condition. Thus, in *Meloidogyne*-infections there are multinucleate giant cells, whereas *H. ivorense* and *R. macrodoratus* induce uninucleate giant cells. This point was considered at a discussion session at the XIIIth International Nematology Symposium (*Nematology News :* 30, 3, 1976) and the consensus was that the term, giant cell, refers only to the size of the cell and not to its nuclear condition.

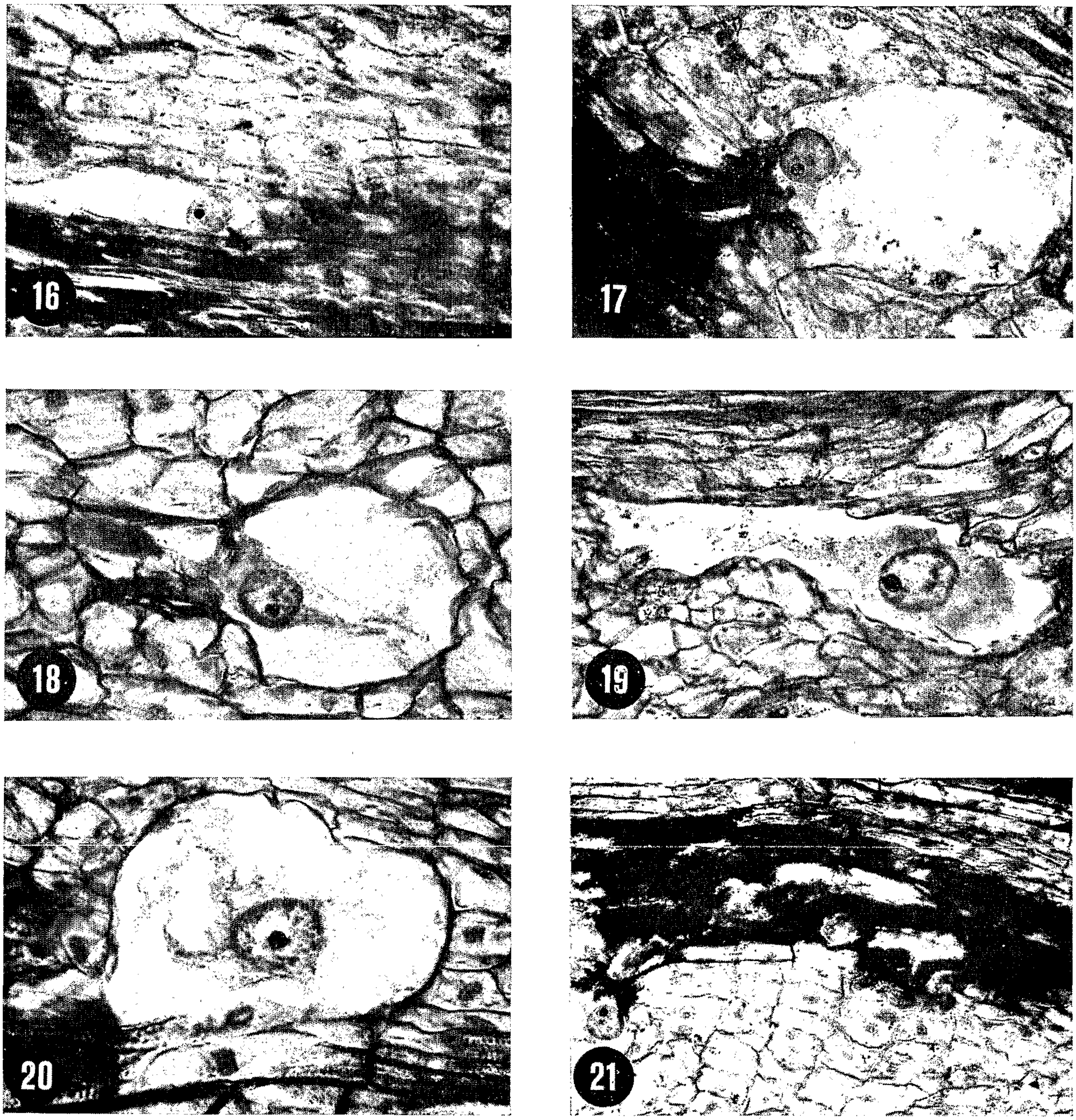
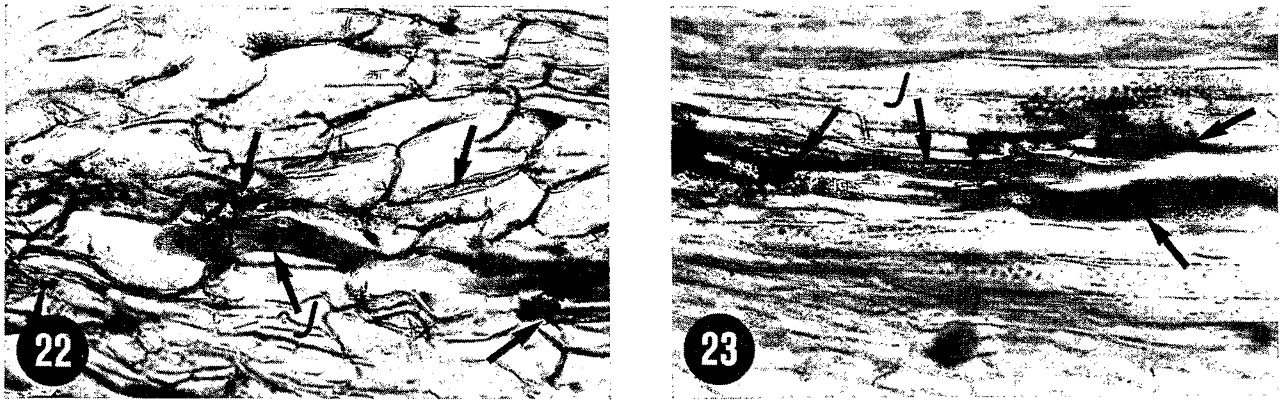


Fig. 16-20 : Giant cell development in *Turraeanthus africana ;* 16 : ten days after inoculation ; 17 : fifteen days ; 18 : twenty days ; 19 : twenty-five days ; 20 : forty days.

Fig. 21 : Severe necrosis around juvenile fifteen days after inoculation.



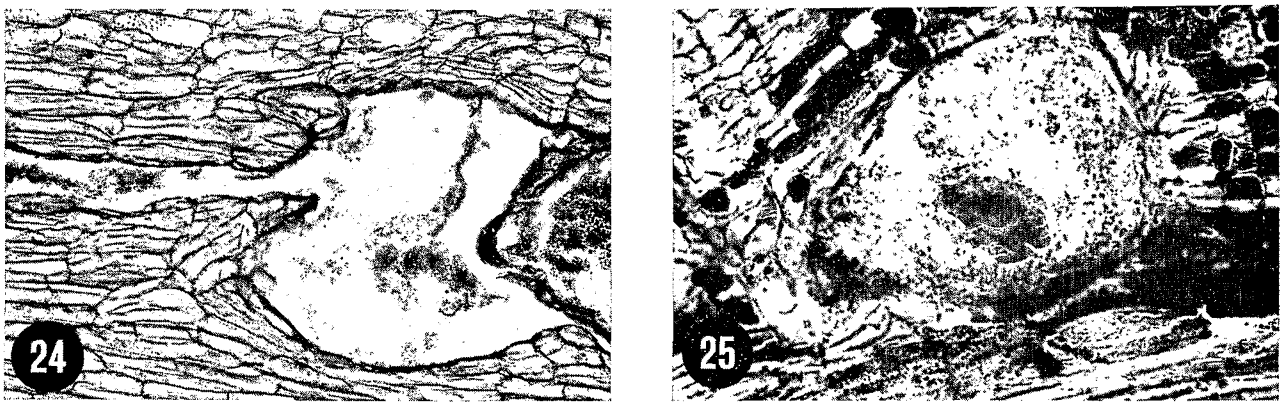


Fig. 22-23 : Necrosis, indicated by arrow near juveniles (J) of *Hylonema ivorense* in roots of *Turraeanthus africana* five days after inoculation ; 22 : moderate necrosis ; 23 : severe necrosis.

Fig. 24-25 : Giant cells in other hosts of *Hylonema ivorense ;* 24 : *Neuropeltis prevosteoides ;* 25 : *Khaya ivorensis.*

This concept has also been proposed by Cohn and Mordechai (1977).

The production of a single, uninucleate giant cell by *H. ivorense* appears nematode-specific rather than host-specific in that similar histopathological effects were observed in *Turraeanthus africana* (Meliaceae), *Neuropeltis prevosteoides* (Convolvulaceae) and *Khaya ivorensis* (Meliaceae). Inoculations of *T. africana* with juveniles of *Meloidogyne* spp. to observe the nuclear condition of giant cells induced by these nematodes have repeatedly been unsuccessful.

The nature and origin of the *Hylonema*-induced giant cell and its enlarged nucleus remain unknown. Our inability to discern a discrete cell wall surrounding the giant cell suggests that it may consist of a naked mass of protoplasm bounded by adjacent cells. Confirmation of this awaits electronmicroscopic examination of infected tissues. Similarly, no conclusion can be made at present regarding the chromosomal situation in the enlarged nucleus.

The large amounts of necrosis in infected roots of *T. africana*, with or without associated fungi, and the probable disruption of translocation caused by the disoriented xylem suggest that *Hylonema ivorense* may adversely affect the growth and timber-producing potential of this tree. *T. africana* occurs in a belt in West Africa from Liberia to Zaïre and its timber, possessing several desirable qualities, has been exploited commercially in Ghana and Ivory Coast. Commercially important damage may also be caused to another host, *Khaya ivorensis* ("African mahogany" or "acajou"), which grows over a much wider range in West Africa and is of greater economic potential.

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