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Histopathology of ginger *(Zingiber officinale)* infected by soil nematode *Meloidogyne sp.*

By

Jaisukh J. Shah and E. Chacko Raju *)

With 23 Figures

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Summary

Histopathology of ginger (*Zingiber officinale* Rosc.) infected by soil nematode *Meloidogyne sp.* has been investigated. Except the aerial shoot system all the other parts of the plants, the roots, rhizomes and scale leaves are infected by the nematodes. The initial target of infection is near the xylem pole. Hyperplasia of parenchyma cells is common in the infected rhizomes and roots. However, galling is conspicuous in adventitious roots. The infection induces the formation of giant cells and the active division of cells surrounding the infected area. The giant cell show akaryotic division of nuclei and thickened cell walls which may show fine irregular wall projections.

Zusammenfassung

Die Histopathologie mit *Meloidogyne sp.* (bodenbewohnende Nematode) infizierter Ingwer-Pflanzen (*Zingiber officinale* Rosc.) wurde untersucht. Mit Ausnahme des oberirdischen Sproßsystems werden alle Teile der Pflanze infiziert (Wurzeln, Rhizome und Blätter). Erstes Ziel der Infektion ist die Nähe des Xylemstranges. Hyperplasie der Parenchymzellen kommt in den infizierten Rhizomen und Wurzeln häufig vor. Die Infektion führt zu Riesenzellen und zu Zellteilungen in dem das Infektionsgebiet umgebenden Gewebe. Die Riesenzellen zeigen amitotische Kernteilungen und verdickte Zellwände mit feinen unregelmäßigen Vorsprüngen.

(Editor)

*) J. J. Shah and E. C. Raju, Department of Botany, Sardar Patel-University, Vallabh Vidyanagar, 388 120, India.

1. Introduction

Amidst the various organisms harbouring the soil, in the rhizosphere of plants, nematodes form a chief taxon and they greatly influence plant growth and crop production. "Root-Knot" nematode as a parasite of edible ginger has been reported from Japan, Queensland, Australia, Hawaii (see HAUNG 1966) and Kerala (S. India) (MAMMEN 1973). Little is known about the pathological anatomy of ginger infected by soil nematode (HAUNG 1966). Hence it was thought worth to investigate this aspect throughly in ginger (*Zingiber officinale* ROSC.), which is one of the most important rhizomatous spices (PARRY 1969). This is the fifth contribution in a series of anatomical studies on some rhizomatous plants (SHAH & RAJU 1975a, b, 1976; RAJU & SHAH 1975).

2. Materials and Methods

Infected parts of ginger-rhizomes and roots were collected from the Sardar Patel University Botanical Garden and were fixed in FAA (SASS 1958). The fixed materials were washed 2—3 times in 70% alcohol and dehydrated in TBA series and embedded in tissuemat (SASS 1958). Sections 5—8 μm thick were stained in Regaud's haematoxylin — fast green or in Safranin O — fast green combinations (GURR 1965). The periodic acid — Schiff's (PAS) reaction (JENSEN 1962) was used as a test for carbohydrates. DNA and RNA were localized by Azure B (JENSEN 1962). For the detection of sieve elements in the infected tissues of rhizomes and roots, the sections were stained with tannic acid—ferric chloride and resorcin blue combination (CHEADLE, GIFFORD & ESAU 1953).

3. Observations

3.1 Visible Morphological Symptoms

The "root-knot" disease of ginger is caused by the nematode, *Meloidogyne* sp. Infection takes place at any stage of development in ginger, although the incidence of infection is greater in the roots of a three months old plant. The first external symptom of infection is the yellowing and drooping of the foliage leaves at the lower nodes (Fig. 1). In the case of severe infection the entire shoot system of the plant collapses and no branching of the underground rhizome occurs. The infected roots show abnormal swelling (Fig. 2, dart). The infected rhizomes show weak growth. They are spongy and become filled with water when soaked for about 2—9 hours.

In the rhizome the outer cells of the inner ground tissue appear infected (Fig. 3). The scale leaves of the rhizome may also show infection (Fig. 4) up to the adaxial and abaxial epidermal cells. The dried mature scale leaf often shows the presence of nematodes. A root at the time of the harvest is heavily infected.

3.2 Site of Infection and Movement of the Nematode in the Host Tissue

Infection is brought about by the young larvae of the nematode. The anatomical changes in the host tissue are induced by the female nematode which is large and rounded with a curved anterior part. A cavity is produced near the posterior and of the female nematode (Fig. 22). The eggs are hatched in this cavity and the larvae migrate to the adjacent regions of the host tissue (Fig. 5). The migration of the larvae is intercellular. In the root they may be found within intercellular spaces (Fig. 6). But even in such cases evidence of cell wall breakage in the vicinity is usually found (Fig. 6, arrows). In majority of the infected vascular bundles of the rhizome and the roots the phloem region remains unaffected (Figs. 10, 20 arrows). In a root some of the xylem parenchyma cells are infected (compare Fig. 21 and 23). These cells transform into giant cells and hence the metaxylem vessel elements are crushed. The above observations show that the initial target of infection is the xylem.

3.3 Effects of Infection

Once the larvae enter the host tissue they develop into adult female or male. The histological changes are brought about by the female nematode. One of the earliest tissue responses following the infection by the female nematode is the division of cells surrounding the infected area (Figs. 3, 11, arrows). The most striking result of infection is the formation of giant cells (Figs. 4, 8, 9, 11, 21). Development of abnormal tracheary cells was observed around the giant cells (Fig. 11, dart). The egg matrix of the nematode appeared to be destructive to the rhizome and root as the cells immediately in contact with it collapse (Fig. 19, arrows). Later the areas occupied by the female nematode, egg matrix and giant cells appear empty (Fig. 13). The empty cavities in a rhizome may show fungal infection.

3.4. Giant Cells

The giant cells are formed near the anterior portion of a resting female nematode (Figs. 8, 22). They show an unusual enlargement, nuclear division without cytokinensis and dense cytoplasm (Fig. 9). The female nematode appears to be stationary near the xylem tissue. Some of the xylem parenchyma cells differentiate as giant cells (compare Figs. 21, 23). Occasionally the common cell wall between a giant cell and parenchyma cells shows breakage (Fig. 7, arrow). Sometimes the remnants of cell wall are seen in the giant cells (Fig. 21, arrows). This probably indicates the involvement of more than one cell in the formation of a giant cell.

The giant cell nuclei and cytoplasm are rich in nucleic acids (Fig. 18). Starch is absent in the giant cell (Fig. 20) and in the cells of the infected region of the rhizome (Fig. 19). Minute protein granules are present in the giant cells. On the outer side of the egg sac a thick layer of insoluble

polysaccharide is present (Fig. 19). A nematode induces the formation of 2—7 giant cells at a time. In a giant cell 8—50 nuclei each with one or more large nucleoli were observed (Fig. 17, 18). They are formed by akaryosis. During its division the nucleus elongates and the nucleolus shows simple fission (Fig. 17, arrow). Later the nucleoli separate and are seen at the two poles of the nucleus (Fig. 17, dart). The nucleus further elongates and becomes stretched in the middle (Fig. 12, dart), and finally it becomes divided into two daughter nuclei (Fig. 12). A large number of unequal nuclei are formed in this manner in a giant cell. At a time more than one nuclei may undergo divisions (Fig. 12). Sometimes even after the separation of the nuclei, a tail like portion of the nucleus may be seen. In majority of the giant cells the nuclei appear aggregated (Figs. 4, 9, 11).

The giant cells show thick cell wall (Figs. 9, 12). The wall thickening is irregular (Fig. 14). The thin part of the wall shows breakage (Fig. 16 arrows). In a few instances the wall of the giant cell in the rhizome showed fine projections in an indefinite pattern (Fig. 15).

4. Discussion

Haung (1966) reported both hyperplasia and hypertrophy of the parenchyma cells in ginger roots and rhizomes following infection by *Meloidogyne incognita*. The hyperplasia of parenchyma cells is not pronounced in monocotyledons which are infected by *Meloidogyne* and so infected areas do not show conspicuous galls (Swamy & Krishnamurty 1971). As a response to nematode attack dicots exhibit pronounced external galling. In the present study hyperplasia of parenchyma is observed in rhizome and roots and galling was conspicuous in adventitious roots and its lateral roots. A number of monocotyledonous plants can harbour *Meloidogyne*. For example *Zea mays* could harbour *M. incognita* that produced the usual histopathological effects (Baldwin & Barker 1970). Again Siddiqui & Taylor (1970) and Siddiqui (1971) could successfully inoculate *M. naasi* into the roots of wheat and oats respectively, in which galls containing 2—8 giant cells arose.

In ginger multinucleate giant cells were observed both in the root and rhizome. The development of coenocyte complexes by nematode infection has been recorded in many plants, and also in plants attacked by insects (Dundon 1962). According to Dundon (1962) the number of nuclei of a coenocyte would correspond to the number of cells that are formed by the fusion of several cells. In tomato roots according to Paulson & Webster (1970) the giant cells form in a tissue at a time when cell walls are easily stretched and it is thus possible that giant cell originates by expansion of a single cell. In the present investigation the remnants of cell was observed in a giant cell indicate that some cells might have fused in the formation of a giant cell.

The multinucleate condition of the giant cell is produced by the accumulation of nuclei from several cells and from divisions without cytokinesis (WALLACE 1963). SWAMY & KRISHNAMURTHY (1971) observed frequent occurrence of dumbbell—shaped and dissimilar nuclei in the coenocyte of *Enterolobium* and they feel that amitotic budding of nuclei could be a method of increase in nuclear number. The above workers were not able to observe these features in *Basella*. The giant cells of the roots and rhizomes observed here show a number of nuclei of various sizes. Amitotic division of the nucleus observed in the present studies shows that amitotic budding is a method of increase in nuclear number. The development of treacheary cells from the parenchyma is observed in the roots of *Enterolobium* and *Basella* infected by *M. javanica* (SWAMY & KRISHNAMURTHY 1971). The extensive development of similar cells around giant cells observed in the infected roots and rhizomes is in agreement with the observation of HAUNG (1966).

Irregularly thickened giant cell walls are reported by BIRD (1962) and PAULSON & WEBSTER (1970). Similar cell walls are reported in the secretory cells of venus fly trap and the mucilage producing cells of *Drosera* (see PAULSON & WEBSTER 1970). GUNNING & PATE (1969) use the term "transfer cells" to those cells which possess ingrowths of wall material and hence having protoplasts with an unusually high ratio of surface to volume. In the present study minute wall ingrowths of some giant cells in the rhizome of ginger are observed. According to GUNNING & PATE (1969) the transfer cells have functions of absorption and/or secretion. The giant cells function as a storage house for the feeding purpose of the female nematode as well as for its young larvae (PAULSON & WEBSTER 1970). Once the female nematode selects a feeding cite it is stationary (JENKINS & TAYLOR 1967). Hence the giant cells which are the main source of nutrient supply have to accumulate all the necessary nutrients. Probably the giant cells with this type of wall ingrowths may help in the absorption of solutes.

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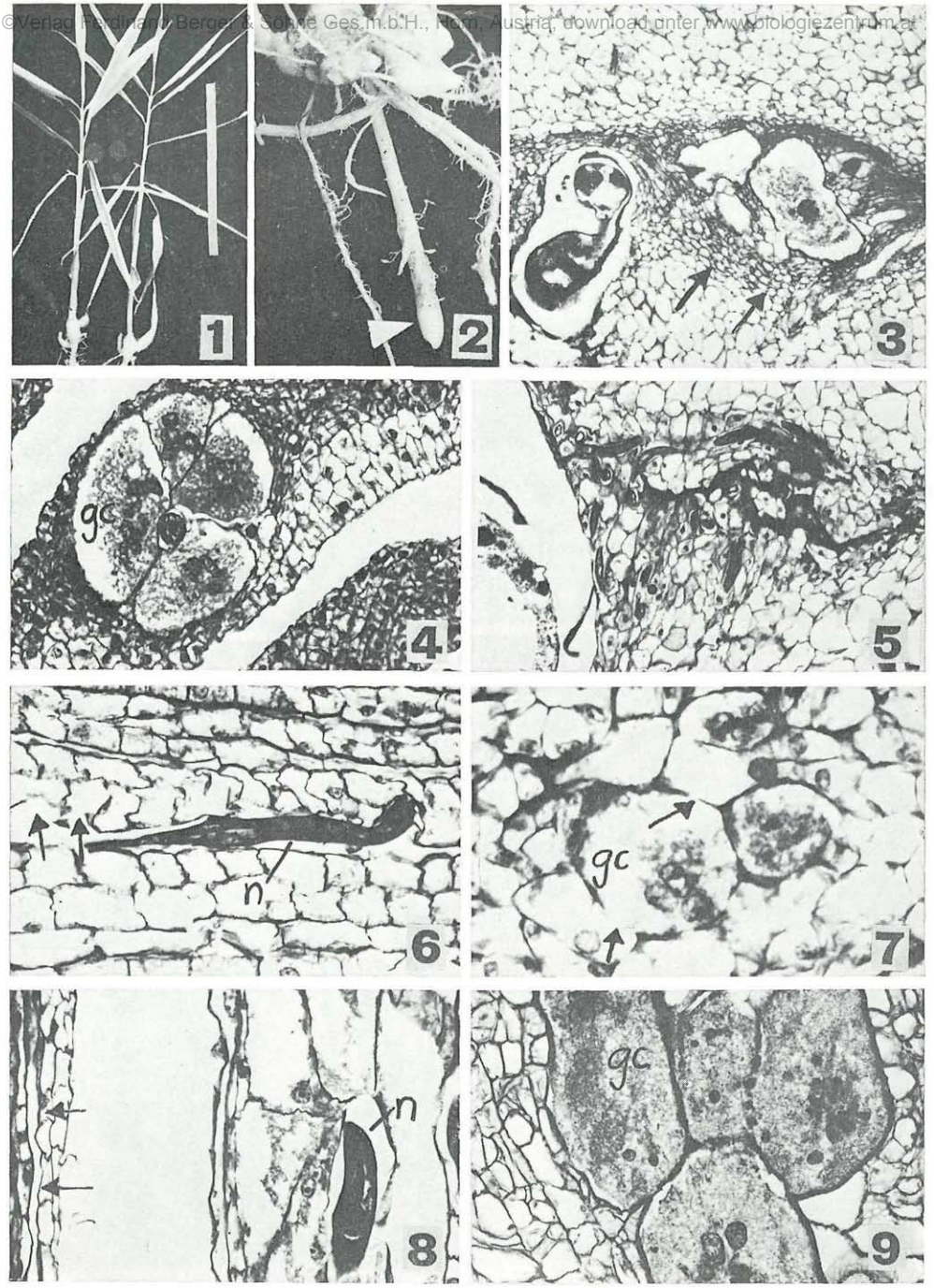
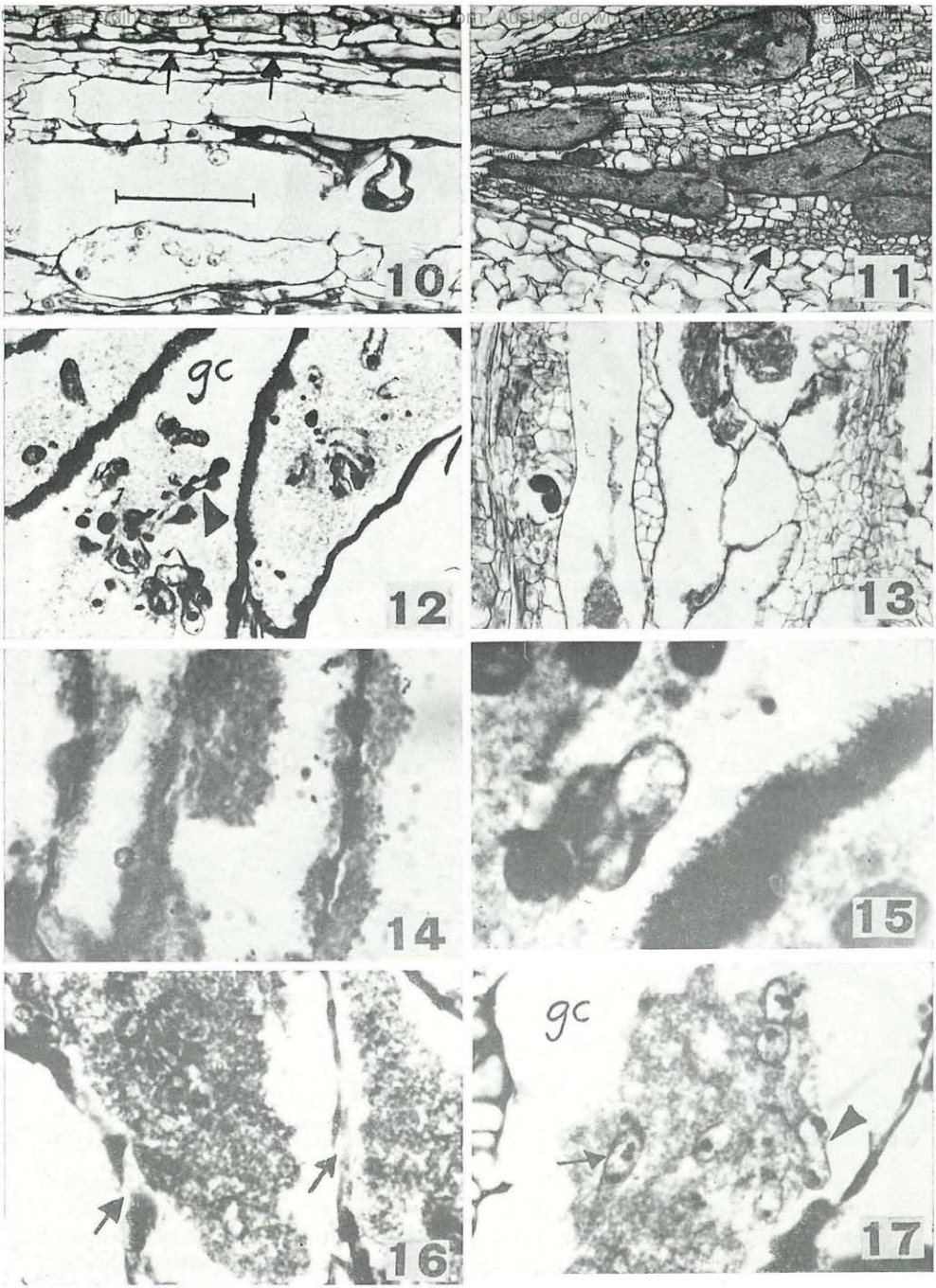
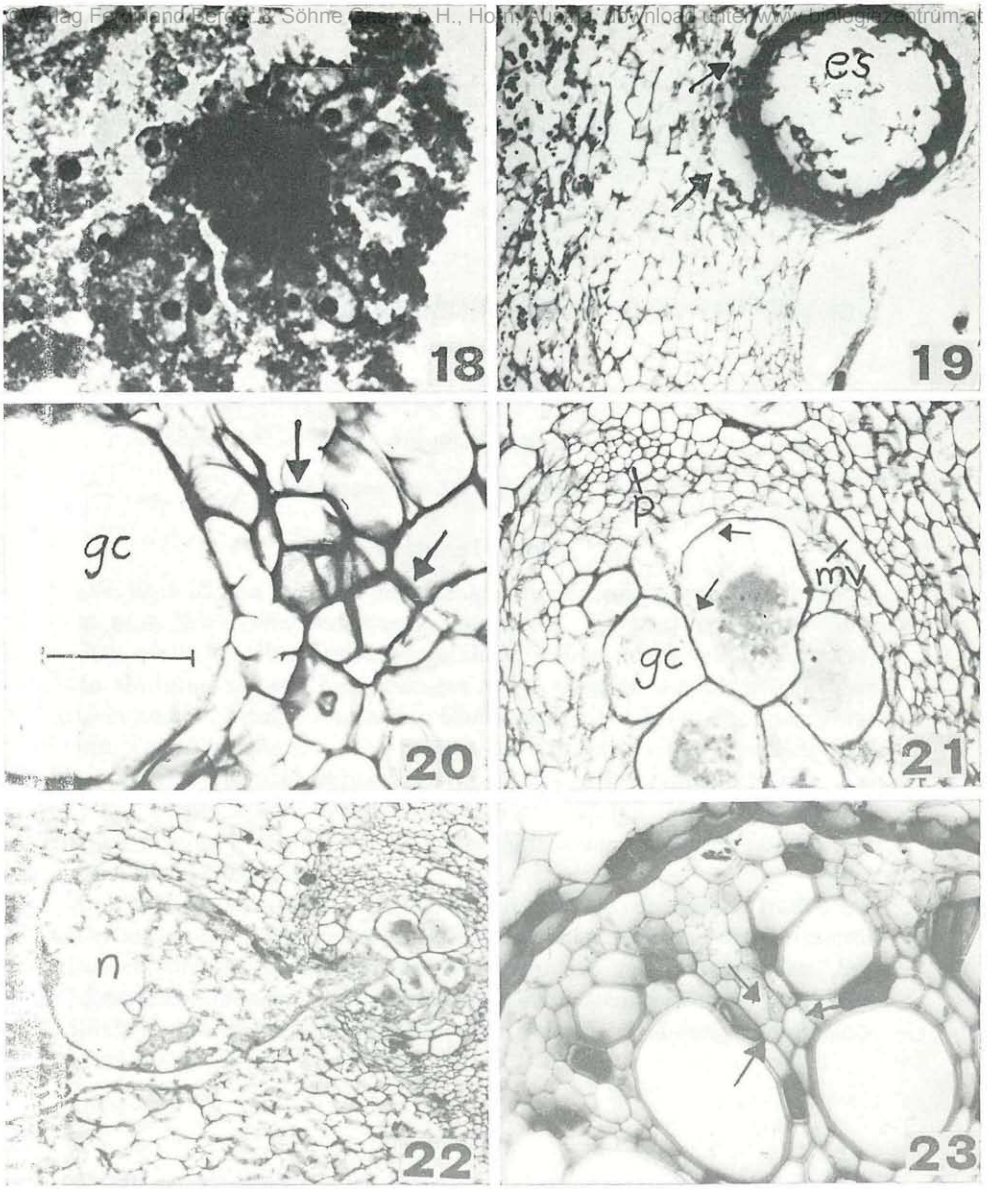


Figs. 1—9. Infected plants and anatomy. (gc = giant cell; n = nematode). — 1. Infected plant with drooping leaves at lower nodes. — 2. Infected swollen roots. — 3. Infected rhizome in transection. — 4. Infected scale leaf. — 5. A group of larvae in the rhizome. — 6. Longitudinal section of a root with a nematode. — 7. Rhizome with young giant cells. — 8. A female nematode with giant cells in a root. — 9. Multinucleate giant cell with abnormal tracheary cells. Scale (inserted in Fig. 1) 1, 2 = 6 cm, 3—5 = 400 μm; 6—9 = 60 μm.



Figs. 10—17. Infected root and rhizome. (gc = giant cell). — 10. Infected root with intact sieve elements (at arrows). — 11. Multinucleate elongated giant cells. — 12. Giant cells of a rhizome with akaryotic nuclear division. — 13. Infected root with cavities. — 14. Unevenly thickened cell wall of a giant cell. — 15. Giant cell wall of a rhizome with fine projections of cell wall. — 16. Breakage of giant cell walls at this region (at arrows). — 17. Giant cell nucleolar fission. Scale (inserted in Fig. 10) 10, 12 = 60 μm; 11, 13 = 400 μm; 14, 16, 17 = 50 μm; 15 = 20 μm.



Figs. 18—23. Infected root and rhizome. (es = egg sac; gc = giant cell; mv = metaxylem vessel; n = nematode; p = phloem). — 18. Giant cell stained with azure B. — 19. Infected rhizome with egg sac, stained with PAS. — 20. Infected rhizome with giant cell and sieve element (at arrow). — 21. Root with giant cells and the crushed metaxylem vessel. — 22. A female nematode with eggs and giant cells. — 23. Transection of a root showing the site of infection (between arrows). Scale (inserted in Fig. 20) 18, 20. = 40 μm; 19, 21, 22 = 300 μm; 23 = 60 μm.