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The Histopathological Reactions of Vigna sinensis to Separate and Concomitant Parasitism by Meloidogyne javanica and Rotylenchulus reniformis

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Abstract: Cellular alterations in cowpea roots and nodules induced by single and concomitant *Meloidogyne javanica* and *Rotylenchulus reniformis* were investigated. *M. javanica* induced giant cells inside the vascular bundles of roots and nodules, and syncytia in cortical tissue of the nodules. In contrast, *R. reniformis* stimulated hypertrophy of pericycle and endodermal cells of the roots and nodules. Syncytia induced in the roots involved a sheet of pericycle cells and an endodermal cell. Cortical cells of nodules also responded to *R. reniformis* infection, initiating wall gaps that led to syncytial formation. Coincidence of giant cells or syncytia of both nematodes was observed either in one vascular bundle or in separate ones. The histopathology of roots and nitrogen nodules infected by the two species remained unique even when they were feeding in close proximity. *R. reniformis* induced characteristic syncytia and *M. javanica* induced giant cells.
Key Words: Giant cells, syncytia, root-knot nematode, reniform nematode, legume, cowpea, bacterial nodules.

Parasitism by *Meloidogyne* spp. on roots (4, 6, 7) and nitrogen nodules (22) involves induction of giant cells from vascular parenchyma; and that by *Rotylenchulus reniformis* Linford & Oliveira on roots includes formation of syncytia, primarily from pericycle cells (13, 18, 19). Walls between *Meloidogyne* giant cells are intact and unevenly thickened (10, 13). The first cell involved in the formation of *R. reniformis* syncytium in the susceptible soybean cultivar Lee is in the endodermis (20). In cowpea, Razak and Evans (18) report that the endodermal cells remain intact, with only slightly thickened walls. Nitrogen nodule invasion by *R. reniformis* has been reported in pigeonpea nodules (2) but no histopathological work has been done.

Coincident infections by *Meloidogyne* sp. and *R. reniformis* have been reported on tomato (17); and its cellular responses to concomitant populations include degeneration of *Meloidogyne* giant cells adjacent to those of *R. reniforms* (16). Other studies on cellular alterations induced on Ladino clover by concomitant *Meloidogyne hapla* Chitwood and *Heterodera trifolii* Goffart (15) or on sugar beet by *M. hapla* and *Heterodera schachtii* Schmidt (11) reveal no interaction between their feeding sites.

These studies were done to compare cellular responses of cowpea (*Vigna sinensis* Endb. var. Fetriat) roots and nitrogen nodules infected with *Meloidogyne javanica* (Treub) Chitwood and *R. reniformis;* and to explore whether interacting effects occur between the feeding sites of these two nematodes coinhabiting the same plant parts.

MATERIALS AND METHODS

Ten-day-old cowpea seedlings grown in 10-cm pots in sterile soil mixed 1:1 with sterile sand were inoculated with suspension of nitrogen-fixing bacteria (*Rhizobium* sp., cowpea group) drenched on the soil surface. One week later, three-fourth of the pots were infested with: 1) two egg-masses of *M. javanica;* 2) fifty egg-masses of *R. reniformis;* and 3) both nematode inocula. All pots were maintained outdoors and watered daily with nitrogen-free nutrient solution (21) for a 30-day period.

M. javanica requires only a few hours for penetration of cowpea roots (9), whereas *R. reniformis* requires several days (14). Therefore, readily formed *M. javanica* galls were subjected to *R. reniformis* infection.

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Healthy nodules, infected roots and nodules, and *M. javanica* galls infected with *R. reniformis* were selected and prepared for histological studies. Specimens were fixed in FAA for at least 24 h and serially dehydrated in ethyl alcohol. After clearing in xylene, they were embedded in paraffin, sectioned (9 μm) longitudinally and transversely, and stained with safranin–fast green (12).

RESULTS AND DISCUSSION

Each of the tested nematodes induced cellular alterations in different tissues of the vascular bundle of the roots. *M. javanica* initiated giant-cell formation in vascular parenchyma (Fig. 1). In contrast, *R. reniformis* caused mild hypertrophy of pericycle cells and endodermal cells (Fig. 2). A ring of hypertrophied pericycle cells was formed

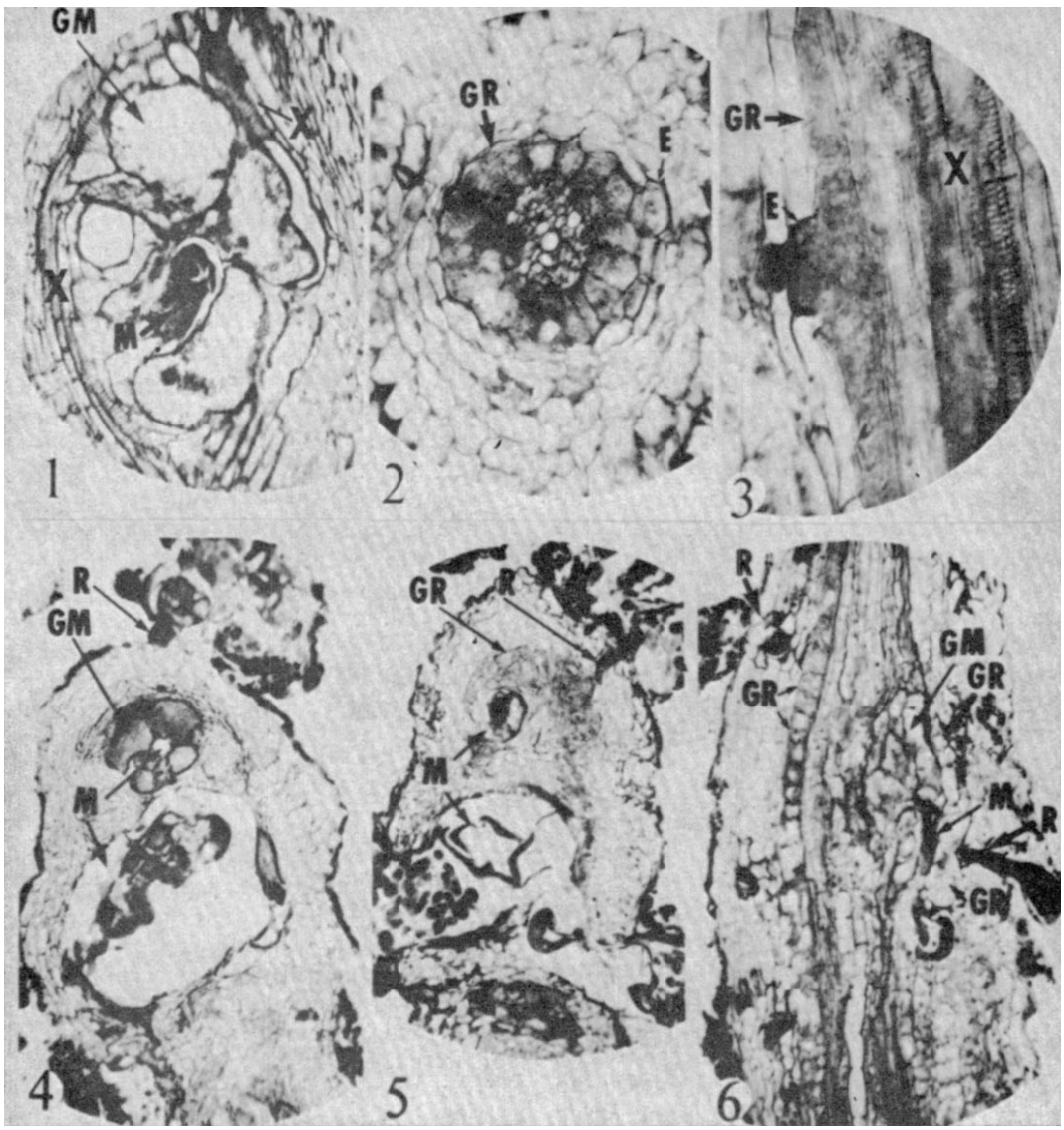


FIG. 1-6. *1)* Longitudinal section of root infected with *M. javanica,* showing the giant cells inside the vascular bundle. *2)* Cross-section of root infected with *R. reniformis,* showing the ring of hypertrophied pericycle cells and an altered endodermal cell. *3)* Longitudinal section of root infected with *R. reniformis,* showing the breakdown of endodermal and pericycle cell walls. *4, 5)* Successive cross-sections of galled root tissue invaded by *R. reniformis,* showing *M. javanica* giant cells (4) and *R. reniformis* hypertrophied pericycle cells (5). *6)* Longitudinal section of root coinhabited by both nematodes, showing their feeding sites. (E = endodermal cell; GM = *M. javanica* giant cells; GR = *R. reniformis* hypertrophied pericycle cells; M = *M. javanica;* R = *R. reniformis;* X = xylem)

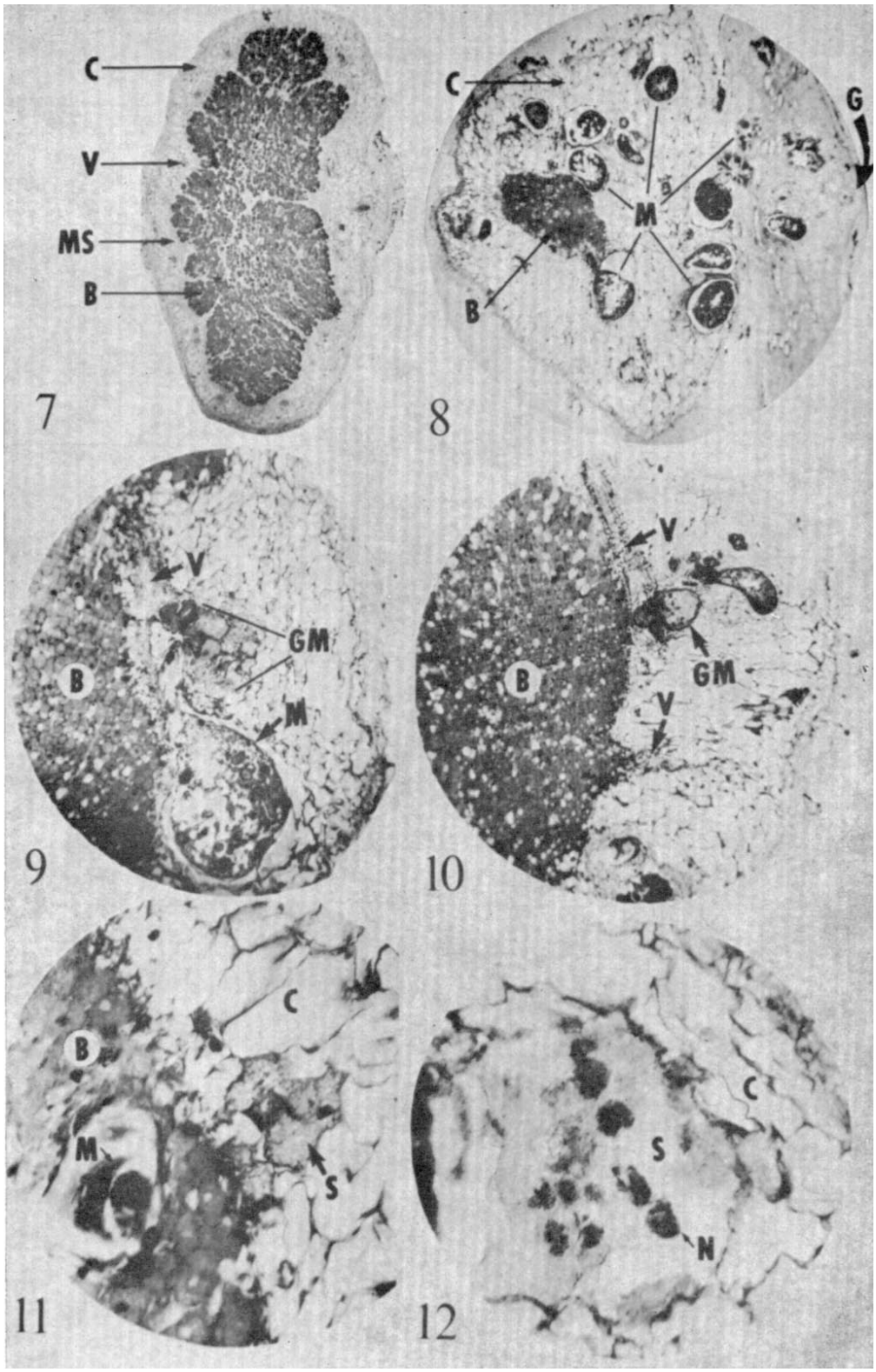
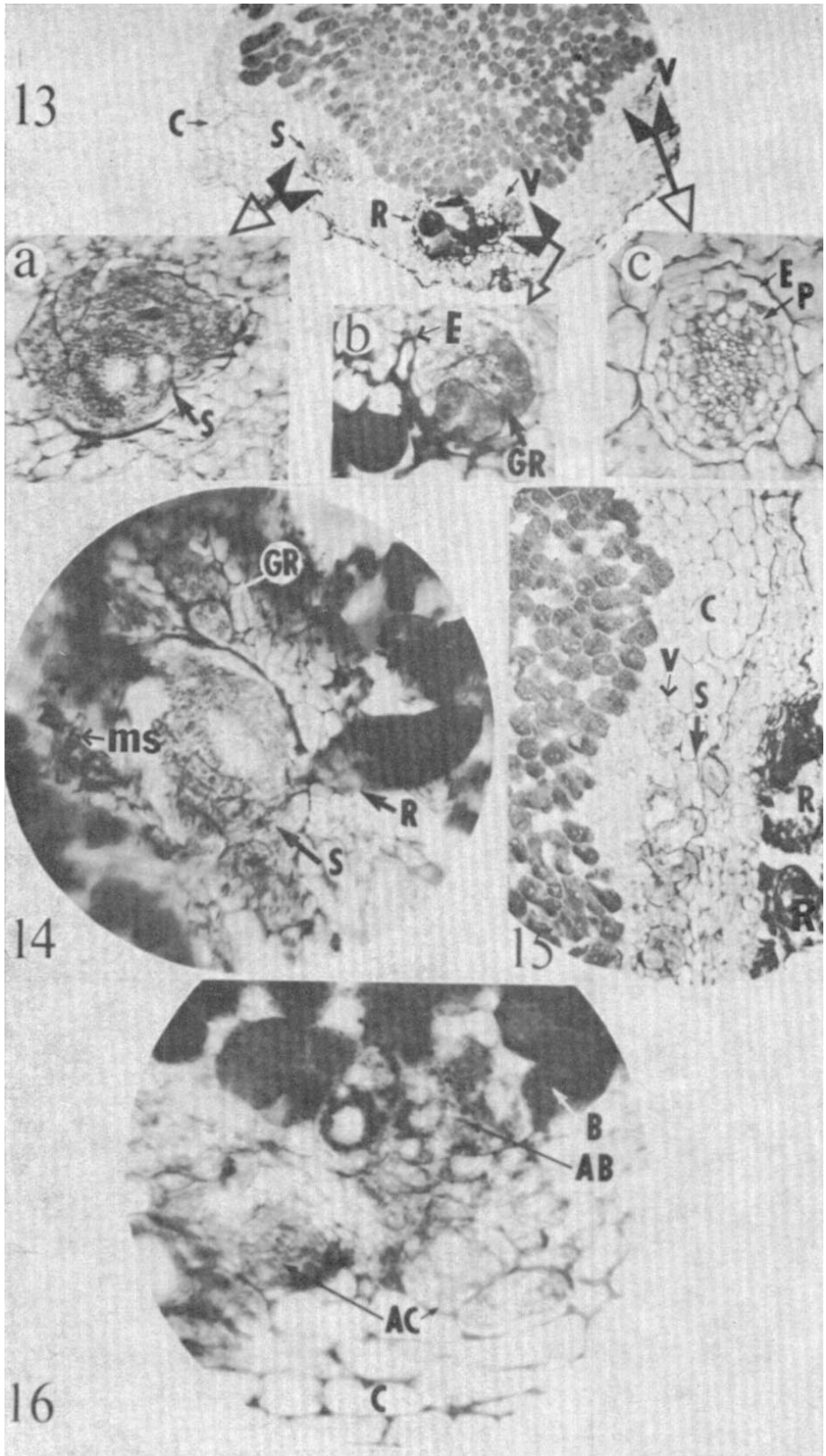


FIG. 7-12. *7)* Cross-section of healthy nodule. *8)* Longitudinal section of nodule infected with *M. javanica* on galled root. *9, 10)* Longitudinal sections through nodule infected with *M. javanica,* showing branched vascular strands within the cortex in which females are embedded. *11, 12)* Cross-sections of nodule infected with *M. javanica,* showing embedded female in bacteroidal tissue (11) and a syncytium in the cortex (11, 12).

(B = bacteroidal tissue; C = cortex; G = gall; GM = giant cells; M = *M. javanica;* MS = meristem layer; N = nucleus; S = syncytium; V = vascular bundle or strands)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as a result of multiple infections by *R. reniformis*. These reactions to these nematodes conform to responses reported earlier by other workers as reviewed by Endo (8).

A breakdown induced by *R. reniformis* of walls between altered endodermal and pericycle cells indicates that both tissues are involved in syncytial formation (Fig. 3). Our observation, agreeing with others on soybean (13, 19, 20) and cowpea (18) is that wall gaps occur in pericycle cells leading to a syncytium. On the other hand, this observation provides no support for the observation that endodermal cells do not take part in syncytial formation on cowpea (18). A thickening of an altered endodermal cell wall which is deeply stained (Fig. 3) in the parasitized region, indicates a thick lignin-type deposit, also observed by earlier investigators (5, 19).

No wall gaps were evident in mature giant cells induced by *M. javanica,* but cell walls were unevenly thickened (Fig. 1, 4, 6). The variable thickness of cell walls has been demonstrated in giant cells induced in purple-seeded broad beans by *M. javanica* (10) and in soybean by *Meloidogyne incognita* (Kofoid & White) Chitwood (13).

Successive sections in galled root tissues invaded by *R. reniformis* showed typical giant cells (Fig. 4) and hypertrophied pericycle cells (Fig. 5). Coincidence of both types of feeding sites without apparent interacting effects was observed in a longitudinal section (Fig. 6). Our observations provide no support for the finding that giant cells adjacent to hypertrophied pericycle cells in tomato roots are empty and devoid of nuclei (16).

The vascular strands in cowpea nodules branch repeatedly within the cortex, forming a network of conductive tissue around the bacteroidal zone (1). These strands could be traced in longitudinal sections (Fig. 9, 10). About 12 vascular bundles were counted in a cross-section (Fig. 7). *M. javanica* females were found embedded in cortical tissue with their anteriors extending into the vascular tissue where giant cells were initiated (Fig. 9, 10). Robinson (22) found *M. javanica* giant cells also in xylem parenchyma of cowpea nodule. Nevertheless, a female was found inside the bacteroidal tissue (Fig. 11), inducing syncytial formation in the cortex (Fig. 11, 12). Christie (4) observed *Meloidogyne* giant cells in the cortex of tomato roots where larvae became established. We observed nitrogen nodules heavily infected with *M. javanica* (Fig. 8), confirming a previous report on Dutch white clover (23).

Nodule conductive tissues were enclosed in a pericycle sheath surrounded by a casperian endodermis (Fig. 13c). A nodule had multiple *R. reniformis* infections, and an endoparasitic female induced hypertrophied pericycle cells extending halfway or more around the conductive tissues (Fig. 13b). Endodermal cells at the site of infection were also altered. Two females of *R. reniformis* were found side by side on the multiple-infected nodule (Fig. 15). One female induced a syncytium consisting mostly of coalesced vascular tissues filled with densely staining cytoplasm and having enlarged nuclei and nucleoli (Fig. 13a, 14). The effect of this nematode extended to include two hypertrophied cortical cells adjacent to the syncytium (Fig. 14), and expansion of the syncytium interiorly caused destruction of the meristem layer. The other female induced a syncytium consisting of cortical cells (Fig. 15). Wall gaps were clearly visible in the altered cortical cells, and these walls were uniformly slightly thicker than the immediately adjacent normal cells. In another case, cortical cells responded to *R. reniformis* infection with a denser cytoplasm although cell identity was retained (Fig. 16). These

FIG. 13-16. Successive cross-sections of nodule infected with three females of *R. reniformis*, showing: *13)* A syncytium consisting of vascular bundle tissues (a), hypertrophied pericycle cells induced by an endoparasitic female (b), and a healthy vascular bundle (c). *14)* Two hypertrophied cortical cells near the expanding syncytium (13a) causing destruction of the meristem layer. *15)* A syncytium induced by another female showing cortical cells with wall gaps. *16)* Cross-section of a nodule infected with *R. reniformis*, showing affected cortical cells and degenerated bacteroidal tissue.

(AB = degenerated bacteroidal tissue; AC = affected cortical cells; B = bacteroidal tissue; C = cortex; E = endodermis; GR = hypertrophied pericycle or cortical cells; MS = meristem layer; R = *R. reniformis;* S = syncytium; V = vascular bundle)

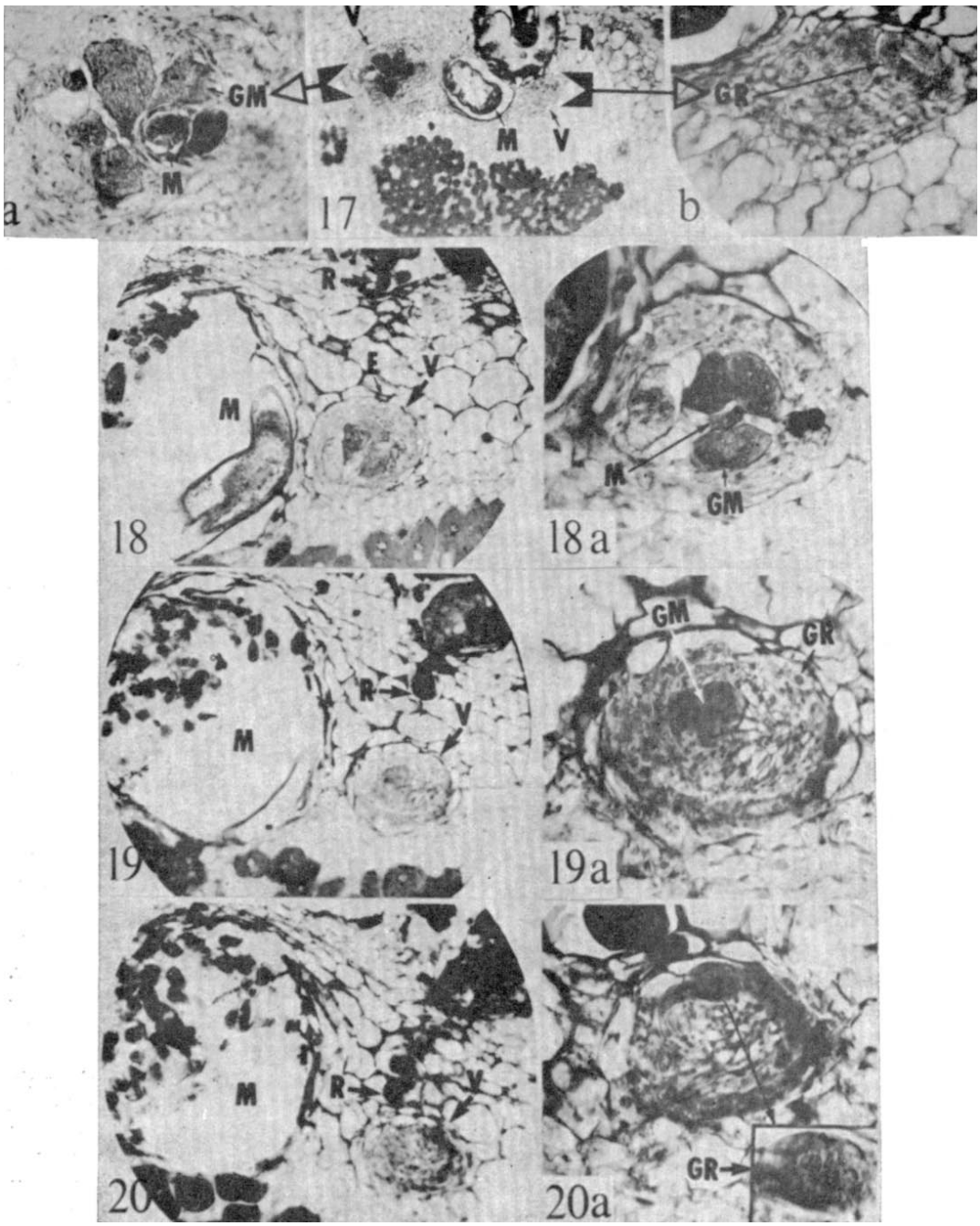

FIG. 17-20. *17)* Cross-section of a nodule coinhabited by both nematodes, showing *M. javanica* giant cells in one vascular bundle (a) and *R. reniformis* hypertrophied pericycle cells in another (b). *18-20)* Successive cross-sections of nodule coinhabited by both nematodes, showing their feeding sites in one vascular bundle:

18a. Giant cells at the infection site of *M. javanica.*

19a. Feeding sites of both nematode species between their infection sites.

20a. Hypertrophied pericycle cells at the infection site of *R. reniformis.*

(E = endodermis; GM = *M. javanica* giant cells; GR = *R. reniformis* hypertrophied pericycle cells; M = *M. javanica;* R = *R. reniformis;* V = vascular bundle)

altered cells may resemble the "nurse cells" induced in citrus roots cortex by *Tylenchulus semipenetrans* Cobb (24). Bacteroidal tissue near the affected cortical cells degenerated (Fig. 16). Birchfield (3) indicated that *R. reniformis* fed throughout the cortical parenchyma in corn with no enlargement of cells.

Coincidence of giant cells (Fig. 17a) and hypertrophied pericycle cells (Fig. 17b) was observed in separate vascular strands of nodules infected with both nematodes (Fig. 17). Furthermore, giant cells and hypertrophied pericycle cells in a single vascular strand were revealed by successive sections of nodule containing both nematodes (Fig. 18, 19, 20). Both types of feeding site appeared together (Fig. 19a) at a point between separate ones (Fig. 18a, 20a).

Each nematode species produced its own histopathological response in either root or nodular tissue, and the response to one species was not affected by the concomitant presence of the other species.

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