Histological Responses of Four Leguminous Crops Infected with *Meloidogyne incognita*

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Abstract: Histological responses to *Meloidogyne incognita* infection in *Rhizobium* nodules of clover, horsebean, lupine, and pea were investigated. The formation of giant cells in vascular bundles of nodules and roots, and the basal connection of the nodule, were usually associated with abnormal xylem and/or deformed xylem strands. However, giant cells did not disturb or prevent the development of nodular tissues. Areas in which galls formed, wall thickness of giant cells, and number of giant cells around the nematode head varied with plant species. Ranking by gall size and giant-cell wall thickness was horsebean > lupine and pea > clover. The multinucleate condition in giant cells resulted from repeated mitoses without subsequent cytokinesis. The resulting nuclei agglomerated in irregularly shaped masses in some giant cells. *Key Words: Meloidogyne incognita,* histopathology, pathogenesis, host-parasite relationship.

Root-knot nematode infection is typically associated with the formation of multinucleate cells that usually develop from vascular parenchyma cells (6) and may cause disruption of xylem elements (4).

Histological effects induced by root-knot nematode in nodular tissues of legumes have been reported in velvet bean (17), Dutch white clover (21), common bean (25), cowpea (11), and peanut (23). Previous studies have shown that *Meloidogyne javanica* and *M. incognita* drastically inhibited nitrogen fixation (22) and nodulation (26) of four leguminous crops: horsebean (*Vicia faba* L., 'Giza 2'), lupine (*Lupinus termis* Forsk, 'Giza 1'), pea (*Pisum sativum* L., 'Little Marvel'), and clover (*Trifolium alexandrinum* L., 'Giza 3'). Total nitrogen content of the shoots of those hosts decreased significantly as nematode infection increased, but total nitrogen content of roots, and nitrogen per g of root or per g of plant was not affected significantly by the *Meloidogyne* infection (22). This research was done to investigate the histological effects of *M. incognita* infections on nodular tissues of the above crops.

MATERIALS AND METHODS

Seeds of the four leguminous crops (clover, horsebean, lupine, and pea) were soaked for a few minutes in a suspension of *Rhizobium* sp., air-dried, and subsequently germinated in sterilized sand in 30-cm-diam pots. Only one seedling was planted in each pot. Inoculum of *M. incognita* (Kofoid & White) Chitwood was obtained from a culture on tomato, *Lycopersicon esculentum* Mill cv 'Commune.' Eggs of *M. incognita* were extracted in a solution of 1% sodium hypochloride (9) and were added to 80 pots at the rate of 25,000 eggs per pot. Another 80 pots served as uninoculated controls. Pots were kept outdoors for 50 days, watered daily with tap water, and once a week with nutrient solution (8). Nematode-free and nematode-infected nodules of each crop were removed and washed thoroughly with tap water. Then they were fixed in FAA for at least 24 h, dehydrated through a series of ethyl alcohol and xylene dilutions, and embedded in paraffin. Sections were cut both longitudinally or transversely at 9 μm thickness and stained with safranine and fast-green (10). About 50 feeding loci in each crop were examined to estimate the area occupied by a gall, numbers of giant cells around the nematode head, area of the giant cell, numbers of nuclei, and the thickness of giant-cell walls.

The nematode-infected nodulated roots of each host were chopped into small pieces which were mixed before taking five 3-g samples. Roots were stained with acid fuchsin in cold lactophenol, and stored in it for not less than 24 h. Stained roots were rinsed in water and shredded carefully with a stout needle to facilitate counting of *M. incognita* life stages. The occurrence rates of larvae and adult females were computed as percentages of the total number of nematodes per g root.

RESULTS AND DISCUSSION

Nodules of the leguminous crops tested in this study had different types of meristem,

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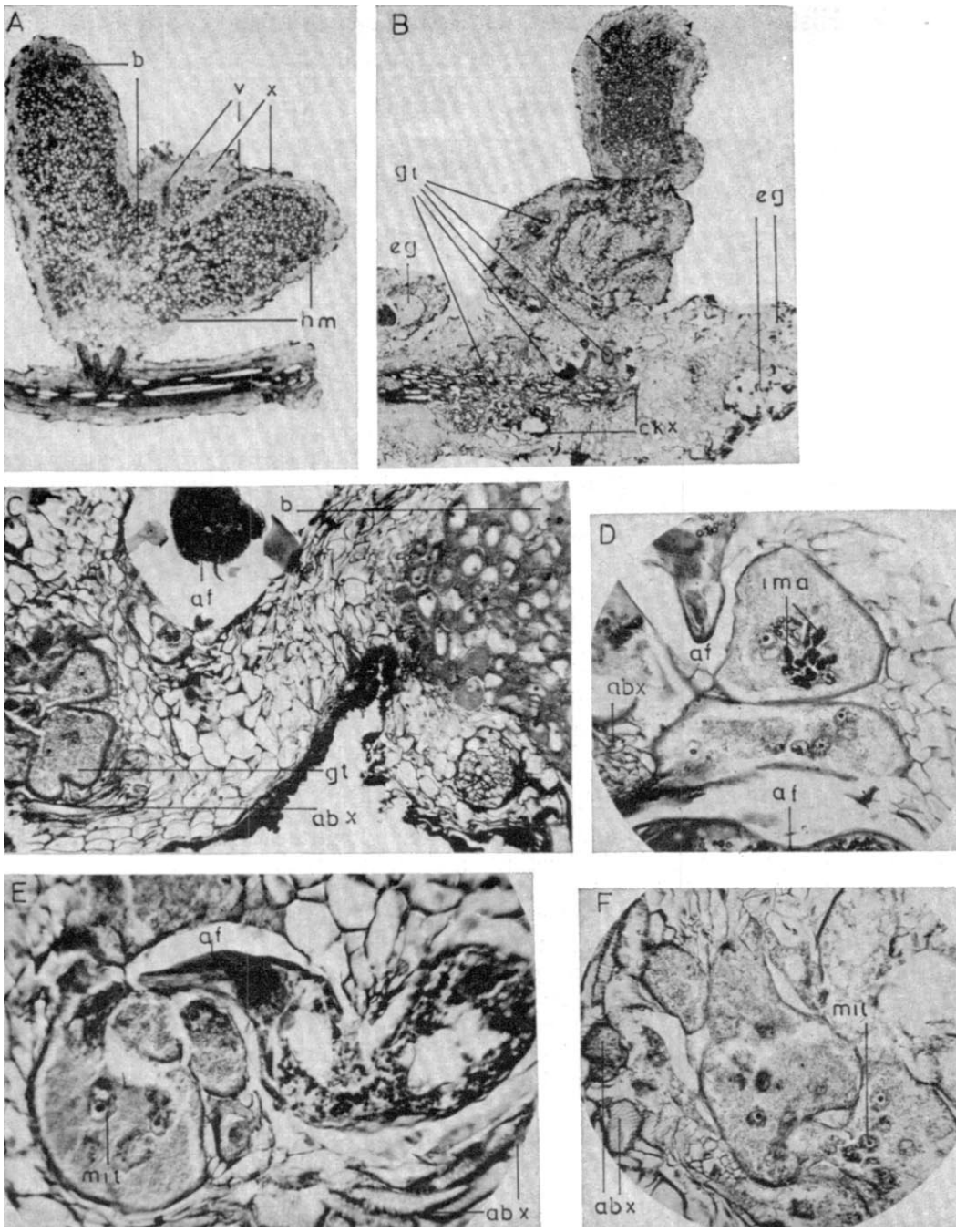

FIG. 1 (A-F). Responses of root and nodular tissues of *Pisum sativum* to *Meloidogyne incognita* infection. A) Longitudinal section of lobed nodule (×15). B) Longitudinal section of infected nodule (×16) showing crooked xylem strands (ckx). C) Adult female associated with giant cells adjacent to bacterial zone (×125) showing abnormal xylem (abx). D) Giant cells in the root (×562.5) showing irregularly shaped masses (ima) and abnormal xylem. E) Giant cells surrounding nematode head in the basal connection of the nodule to the root (×562.5) showing mitosis (mit) and abnormal xylem. F) Development of giant cells in the root (×562.5) showing mitosis and abnormal xylem. (af = adult female; b = bacterial tissue; eg = egg mass; gt = giant cell; hm = hemispherical meristem; v = vascular bundle; x = cortex).

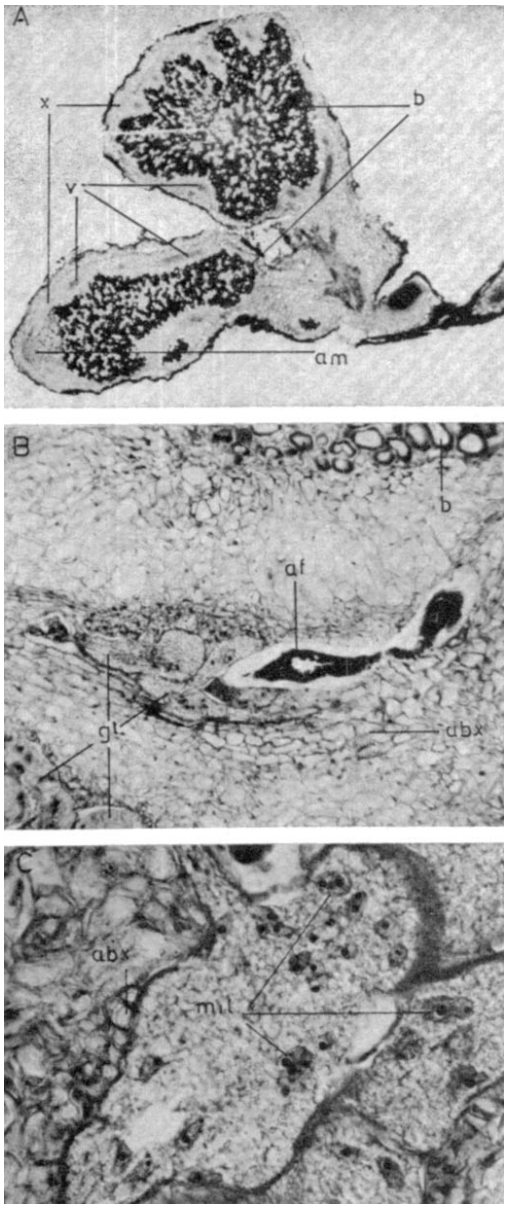

FIG. 2 (A-C). Responses of root and nodular tissues of *Trifolium alexandrinum* to *Meloidogyne incognita* infection. A) Longitudinal section of healthy branched nodule (×24). B) Adult female associated with giant cells adjacent to bacterial zone (×125) showing abnormal xylem (abx). C) Giant cells in the root (×562.5) showing mitosis (mit). (af = adult female; am = apical meristem; b = bacterial tissue; gt = giant cell; v = vascular bundle; x = cortex).

as reported by Allen and Allen (1). The meristem of pea nodule was hemispheric or bowl-shaped, and produced spherical or ovoid nodules as observed in previous studies (3). The occurrence of lobed or irregularly shaped nodules in pea resulted from the uneven or interrupted meristematic activity (Fig. 1-A). Apical meristems (Figs. 2-A, 3-A) produced the elongate, cylindrical nodules common on clover and horsebean. However, unequal growth, or bifurcation, in this type of meristem produced the branched nodules characteristic of clover (Fig. 2-A). Divided lateral meristems are responsible for the nodule formed on lupine (Fig. 4-A). Horizontal growth in opposite directions within these nodules through hypertrophy tended to surround the parent root (Fig. 4-A). The bacteroidal zones become separated by a broad band of parenchyma, as observed earlier (20).

Meloidogyne incognita developed and reproduced in nodular tissues of all the tested crops (Figs. 1-C, 2-B, 3-B, 4-B). The nematodes were found in the vicinity of vascular bundles. As in previous investigations (2, 21), we found that the root-knot nematodes did not alter the structural details of *Rhizobium* nodules in spite of the presence of giant cells. These were located within the vascular strands in the nodules, roots, and the basal connection between the nodule and the root. Vascular bundles occasionally contained both giant cells and female nematodes. As observed in previous studies (12, 16, 18), the pathological reactions included abnormal xylem located in vascular tissues around giant cells. Furthermore, the root xylem strands had the appearance of oblique lines (Figs. 1-B, 3-C, 4-C). This tissue probably resulted from stimulation by nematode feeding or injury to xylem parenchyma.

The efficiency of the symbiotic relationship between leguminous plants and *Rhizobium* is dependent on an adequate vascular system. This relationship is clearly affected by the disruption by nematode infection of the nodule's vascular connection with the root stele. Equally significant was disruption of vascular tissue of main roots by the nematode.

In all the tested hosts giant cells consisted of thickened unbroken walls, dense cytoplasm, and clusters of nuclei. The average area occupied by each gall and the average area of a giant cell was significantly larger in horsebean than in the other hosts except lupine (Table 1). Also, giant

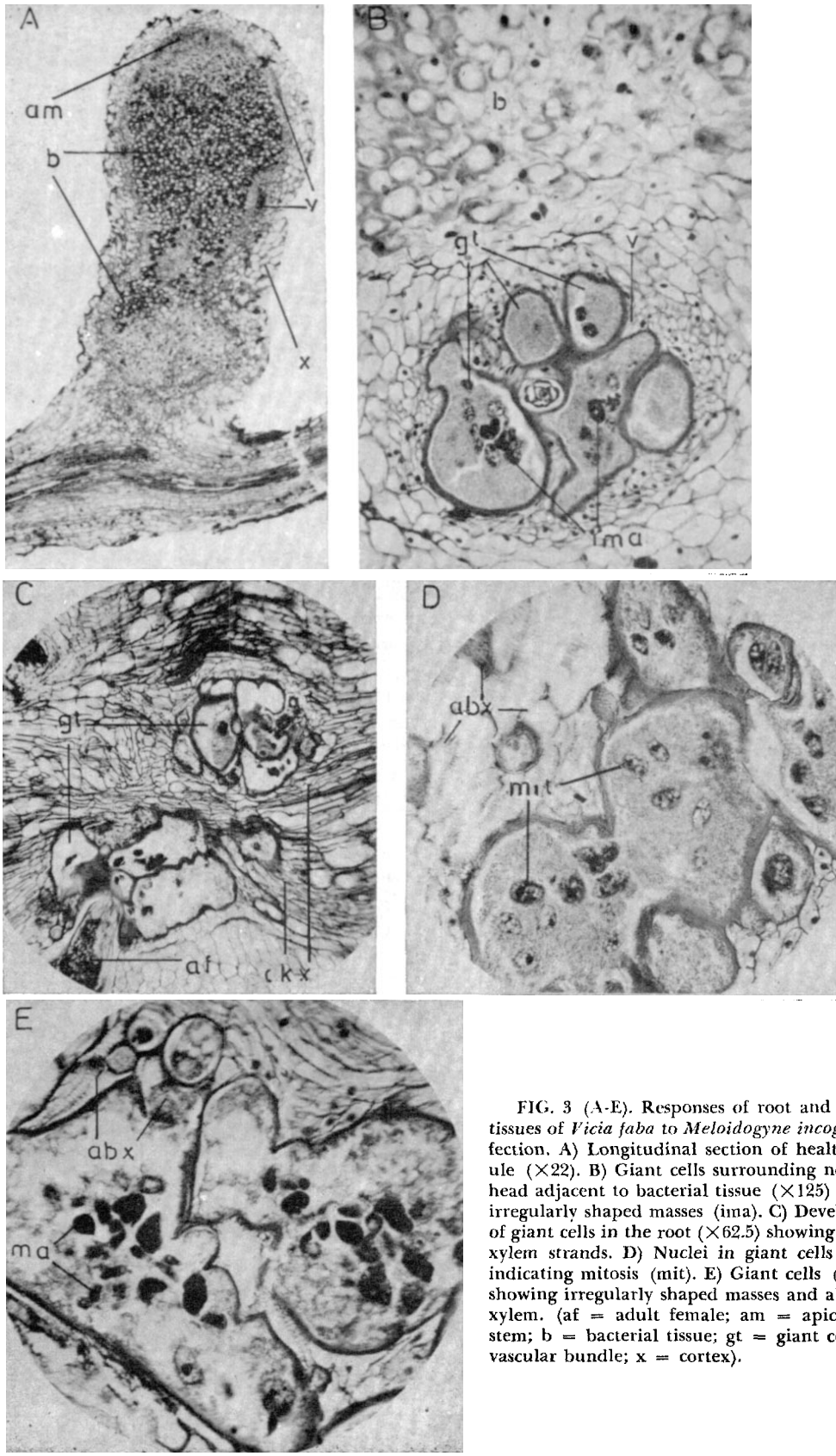

FIG. 3 (A-E). Responses of root and nodular tissues of *Vicia faba* to *Meloidogyne incognita* infection. A) Longitudinal section of healthy nodule (×22). B) Giant cells surrounding nematode head adjacent to bacterial tissue (×125) showing irregularly shaped masses (ima). C) Development of giant cells in the root (×62.5) showing crooked xylem strands. D) Nuclei in giant cells (×400) indicating mitosis (mit). E) Giant cells (×562.5) showing irregularly shaped masses and abnormal xylem. (af = adult female; am = apical meristem; b = bacterial tissue; gt = giant cell; v = vascular bundle; x = cortex).

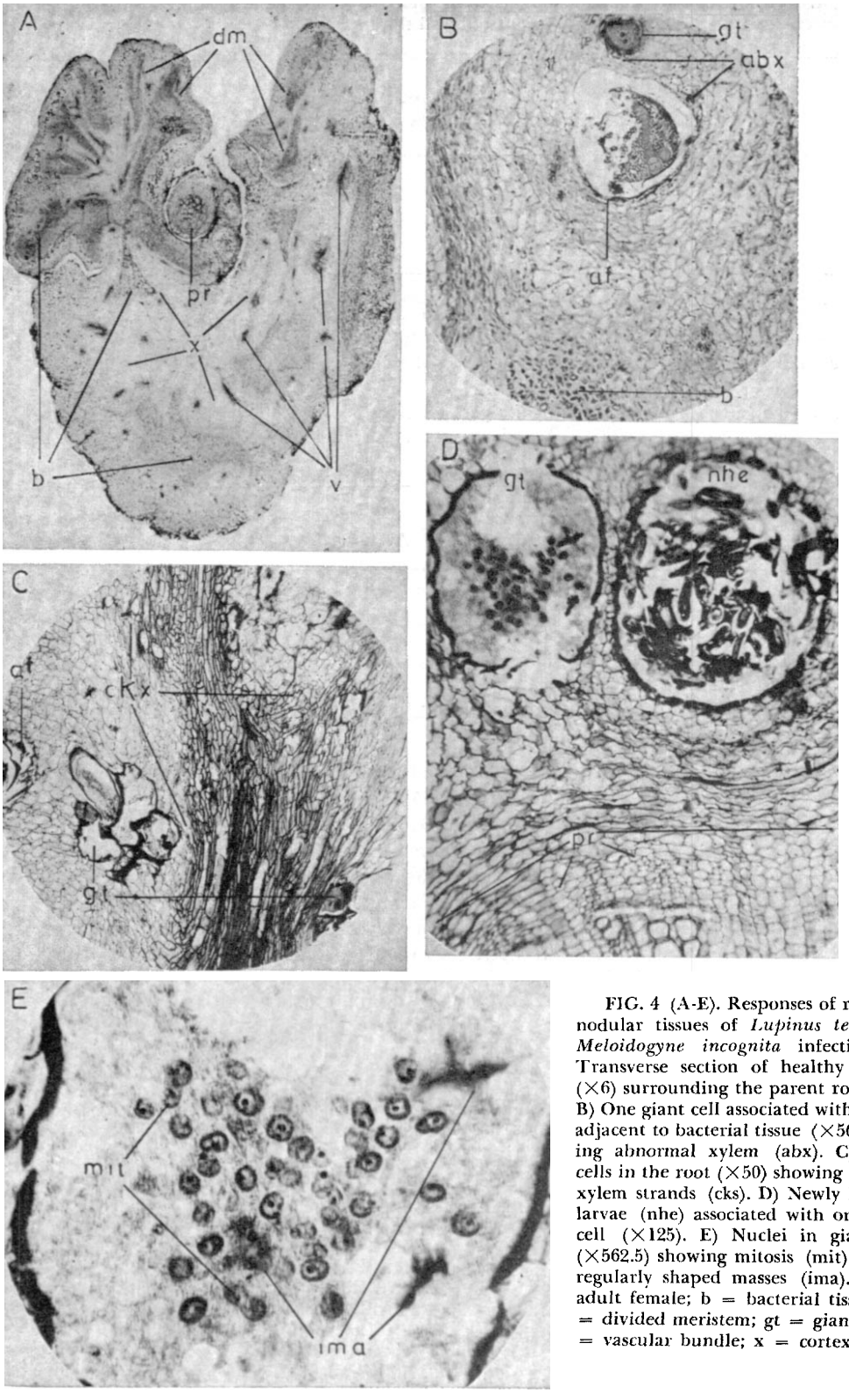

FIG. 4 (A-E). Responses of root and nodular tissues of *Lupinus termis* to *Meloidogyne incognita* infection. A) Transverse section of healthy nodule (×6) surrounding the parent root (pr). B) One giant cell associated with female adjacent to bacterial tissue (×50) showing abnormal xylem (abx). C) Giant cells in the root (×50) showing crooked xylem strands (cks). D) Newly hatched larvae (nhe) associated with one giant cell (×125). E) Nuclei in giant cell (×562.5) showing mitosis (mit) and irregularly shaped masses (ima). (af = adult female; b = bacterial tissue, dm = divided meristem; gt = giant cell; v = vascular bundle; x = cortex).

TABLE 1. Response of four leguminous hosts to *Meloidogyne incognita* as measured by gall size, numbers of giant cell/gall, size of giant cell, and giant cell wall thickness.

Hosts	Gall size[a] (μm^2)	Giant cells per gall[a]	Giant cell size (μm^2)[b]	Giant cell wall thickness (μm)[b]
Clover	15180m	4.7r	3230u	2.5y
Horsebean	71151n	5.4r	13275v	11.7z
Lupine	38195no	2.5s	11862v	8.0z
Pea	31434o	5.7r	5554w	8.0z

[a]Average gall size and numbers of giant cells/gall were evaluated from 50 feeding loci of each host.
[b]Average of giant cell size and cell wall thickness were evaluated from 100 giant cells in each host.
In each column, means not followed by the same letter differ from one another (m-o, n-o, and y-z at the 5% level; and m-n, m-no, r-s, u-v, u-w, and v-w at the 1% level).

TABLE 2. Numbers of *Meloidogyne incognita* per g of roots in four leguminous hosts.[a]

Hosts	Immature stages		Adult females		Number of *M. incognita* per g root
	Av. nos.	Percentage occurrence %	Av. nos.	Percentage occurrence %	
Clover	161	69.3m	71	30.7m	232m
Lupine	239	57.2mn	179	42.8mn	418n
Pea	175	34.6no	330	65.4no	504n
Horsebean	15	3.3o	438	96.7o	453n

[a]In each column, means not followed by the same letter differ from one another (m-no, m-o, and mn-o at the 5% level; and m-n at the 1% level).

cells in horsebean had the thickest cell walls while that of clover was significantly the thinnest one ($P = 0.05$). Galls in pea (31,434 μm) were about half the size of galls in horsebean (71,151 μm). Although giant cells were twice as large in lupine as in pea ($P = 0.01$), cell wall thickness did not differ. The number of giant cells associated with nematode head were fewer in lupine ($P = 0.01$) than in galls formed in other hosts (Table 1, Fig. 4-B,D). The development of small galls and giant cells with thinner cell walls in clover was correlated with lower ($P = 0.01$) numbers of *M. incognita* and the lowest percentage of adult females in the roots of that plant (Table 2), indicating that clover is a less suitable host for this nematode than the other plants tested. Although the nematode populations attained in roots did not differ significantly between horsebean, pea, and lupine, horsebean had significantly more adult females and significantly fewer immature stages than clover and lupine.

Like previous workers (13, 24), we found that nuclei of giant cells were large and had large nucleoli (Figs. 1-E,F, 2-C, 3-D, 4-E). The multinucleate condition of giant cells has been shown to be caused by repeated mitosis without cytokinesis (7). Our observations indicate irregularities in size and shape of giant cell nuclei. They appear to be irregularly agglomerated and form irregularly shaped masses in some giant cells (Figs. 1-D, 3-B,E, 4-E). While it was difficult to count the exact numbers of nuclei in each giant cell, our estimates reveal variable numbers even in the same host. In giant cells induced by *M. incognita* we counted 25 to 54 in clover, 17–66 in pea, 25–80 in lupine, and 21–71 in horsebean.

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