

## Autoradiography of Developing Syncytia in Cotton Roots Infected With Meloidogyne incognita<sup>1</sup>

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*Abstract:* Cotton (*Gossypium hirsutum*) seedlings, uniformly infected with *Meloidogyne incognita*, were exposed for periods of 1-15 days to a nutrient solution containing tritium-labelled thymidine. Syncytium formation began with the amalgamation of cells near the nematode head, and was followed by synchronized mitoses of the nuclei which had been incorporated into a single cell. Syncytial nuclei synthesized DNA in roots harvested 3, 6, 9, 12, and 15 days after inoculation. Seedlings transferred from unlabelled to labelled nutrient solution 9 days after inoculation, and grown for 6 more days, contained some syncytial nuclei which did not become labelled. Giant-cell nuclei increased in size and, in many cases, all nuclei in one giant cell of a set showed active DNA synthesis at about the time the nematode molted to the adult stage. *Key Words:* root-knot nematode, giant cell, DNA synthesis.

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Giant cells are a unique host response to feeding by plant-parasitic nematodes of the family Heteroderidae. They were first described for *Meloidogyne* sp. by Treub in 1886 (4), and have since been the subject of many detailed studies.

There is general agreement that giant cells induced by *Meloidogyne* Goeldi begin with

the swelling of a few nuclei in the pericycle and other undifferentiated root tissues near the head of an entering second-stage larva. As the larva develops, 2-12 giant cells are formed which are characterized by thickened cell walls, dense granular cytoplasm, and numerous hypertrophied, polyploid nuclei.

The origin of the multinucleate condition, however, has not been clearly determined. Most workers (3, 4, 6, 9, 11) have described evidence of cell wall dissolution and coalescence of adjacent cells. Huang and Maggenti (7), on the other hand, suggested that cell wall dissolution plays no part in syncytium formation, and that multinucleosis arises through repeated endomitoses within a

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single cell. Their conclusions were based upon the observation that in *Vicia faba* L., chromosome number in a giant cell followed the geometric progression 12, 24, 48, 96, and 192 and that such numbers could be derived only by divisions starting from a single nucleus. Omitted from this hypothesis were the facts that nuclear membranes were not apparent at metaphase or anaphase (when the chromosome counts were made), and their own observation that within a giant cell individual metaphase plates may possess different numbers of chromosome sets. Thus, for example, 192 chromosomes in a single cell could be attributed to the fusion of nine adjacent cells, seven of which contained tetraploid nuclei and two of which contained diploid ( $2n = 12$ ) nuclei. Similarly, 48 chromosomes per giant cell could have arisen by fusion of two diploid cells and one tetraploid cell. Alternatively, following fusion of odd numbers of cells, their nuclei could independently become polyploid through endo-reduplication (11). However, fusion of cells does not account for the apparent lack of giant cells containing intermediate numbers of chromosomes.

Incorporation of tritium-labelled thymidine by giant cell nuclei has been demonstrated by Rubenstein and Owens (13). They concluded that these nuclei actively synthesize DNA, and that DNA synthesis within a syncytium was dependent upon the close association of a feeding nematode. Label was applied, however, under conditions which did not permit close correlation of DNA synthesis and nematode development, and no effort was made to determine the origin of the labelled nuclei.

Our purpose was to follow the incorporation of tritiated thymidine into giant cells induced in cotton roots by *M. incognita* (Kofoid and White) Chitwood, and to assess the patterns of incorporation in terms of nematode development and syncytial formation.

### MATERIALS AND METHODS

Cotton seedlings (*Gossypium hirsutum* L., 'M8') were inoculated with second-stage *Meloidogyne incognita* larvae by methods described previously (10). Briefly, the seeds were germinated in rolls of germination paper and seedlings were placed in boxes of vermiculite with their root tips between 1-cm-wide strips of Miraclath®. Larvae from eggs

hatched in Aretan® were added to the Miraclath strips. After a 24-h exposure to the larvae, the infection site on each seedling was marked with a loosely knotted silk suture, and the seedlings were removed from the Miraclath and transferred to plastic racks in glass chromatography tanks containing complete nutrient solution. Infection at the site marked by the silk suture was thereby limited to the 24-h period during which the roots were between the Miraclath (10). Each of the two tanks contained 1.0 liter of nutrient solution and six racks, each holding five seedlings. Tank 1 also contained 1.0 mCi of <sup>3</sup>H-methyl-thymidine. One rack was removed from the labelled solution at intervals of 24 and 48 h after the end of the inoculation period, and every 3 days thereafter for 15 days. Each time a rack of seedlings was removed from the labelled solution, it was replaced by a rack of seedlings from the unlabelled solution (Fig. 1). Solutions were constantly aerated with humidified air and changed every 4 days.

At harvest, the roots were washed and the 1-cm infection site was excised, washed in running tap water for 1 h, and fixed in acetic acid-ethanol (1:3, v/v). After all treatments

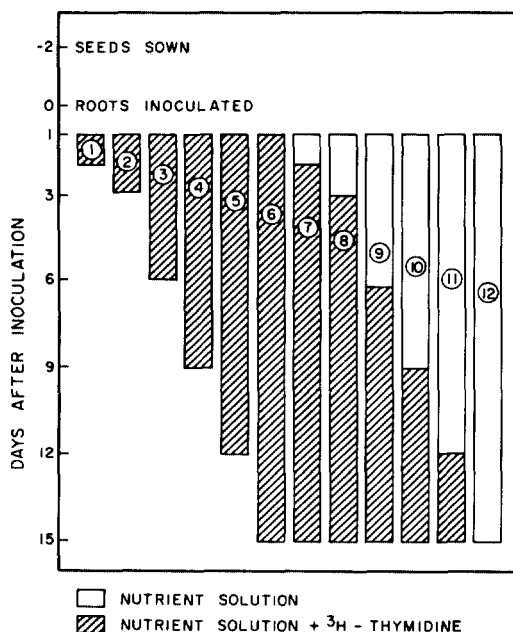
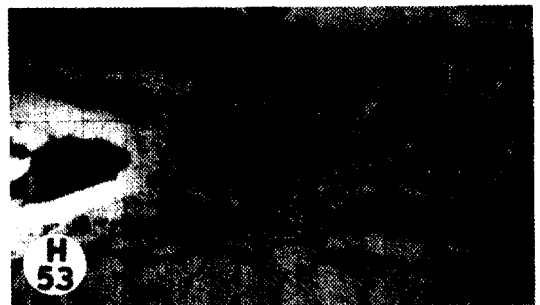
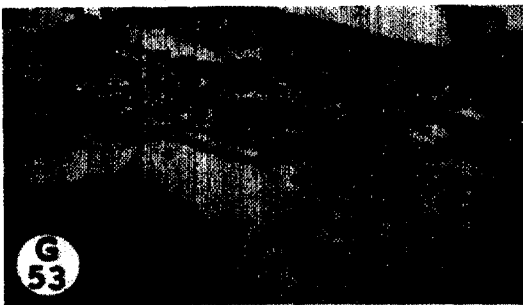
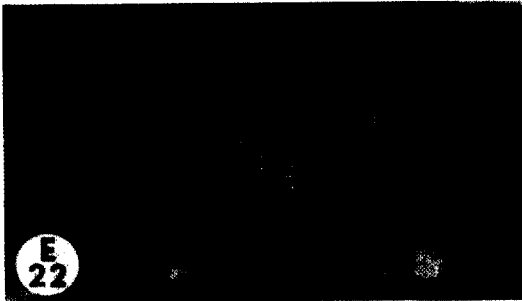
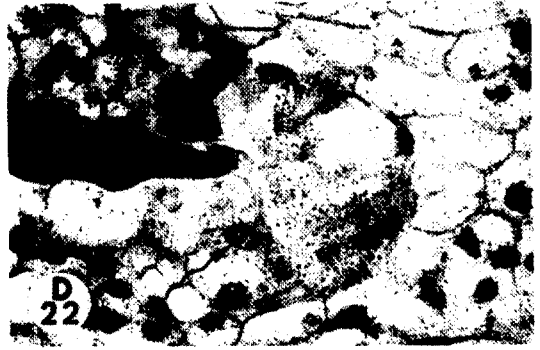
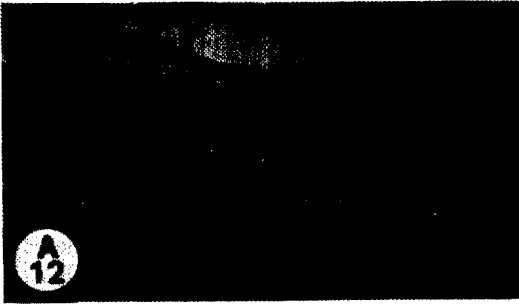


FIG. 1. Experimental design for exposure of cotton seedlings inoculated with *Meloidogyne incognita* to solutions of <sup>3</sup>H-thymidine. Treatment numbers are in white circles.



were harvested, fixed roots were dehydrated through a tertiary butyl alcohol series, embedded in paraffin, sectioned longitudinally at 10  $\mu\text{m}$ , and mounted on slides coated with gelatin-chrome-alum (gelatin, 5 g; chromium potassium sulfate, 0.5 g; water, 1,000 ml). Slides were deparaffinized in xylene and absolute ethanol, air-dried, and dipped in melted (60 C) Kodak NTB2 nuclear track emulsion diluted with an equal amount of distilled water. After being dried, the dipped slides were stored for 2-4 weeks at 5 C in light-tight boxes containing anhydrous  $\text{CaSO}_4$ .

Exposed slides were developed in Dektol (Kodak) (1:1 with water) for 2 min, rinsed, fixed for 5 min, and rinsed in distilled water. Developed slides were stained in basic fuchsin-indigo carmine. Slides stained before dipping in emulsion were unsatisfactory.

## RESULTS

Very early stages of giant cell development were found within 48 h of inoculation. Invading larvae entered the area of the developing stele where many cells were actively dividing and heavily labelled. There was evidence of cell-wall dissolution near the head of the larvae, and of incorporation into a single cell of labelled or unlabelled nuclei with greatly enlarged nucleoli (Fig. 2-A). In some multinucleate cells, all nuclei were undergoing mitosis (Fig. 2-B).

Three days after inoculation, serial sections of enlarged cells near the larval head revealed 1-6 nuclei which were lightly labelled compared to nuclei of surrounding cells (Fig. 2-C). These nuclei were the same size as those of adjacent meristematic cells, but had greatly enlarged nucleoli, a characteristic of developing giant cells (1).

Six days after inoculation, larvae were swollen and giant cells had become filled with

dense, yellow-staining cytoplasm which set them off from surrounding tissues. Nuclei in syncytia were lightly labelled with accumulation of the labelled thymidine in clumps around the periphery of the nucleus (Fig. 2-D,E). Adjacent parenchyma cells appeared to be breaking down and coalescing with giant cells.

Second-stage larvae were greatly enlarged, but had not molted 9 days after inoculation. At this time, the giant cell complex was nearly full-size and syncytial walls were beginning to thicken. Some giant-cell nuclei were labelled more heavily than others, and many had two swollen nucleoli. Surrounding tissues were hypertrophied, and their nuclei were heavily labelled.

All molts had been completed by 12 days after inoculation. The adult stage was accompanied by a doubling in size of syncytial nuclei (Fig. 2-D,E,F,G). Background label in the dense syncytial cytoplasm was higher than that of surrounding tissues.

Young females were swollen, and development of the ovary was well under way, when the final harvest was made 15 days after inoculation. No incorporation of labelled thymidine was noted in developing oocytes, or in any other parts of the nematode. The amount of label varied between giant cells of a group, but appeared to be uniform within individual giant cells (Fig. 2-G).

Treatments 7 through 12 were ended at 15 days. Young females had begun to enlarge, and giant cells were at maximum size. Seedlings placed in labelled nutrient solution at 2, 3, and 6 days after inoculation showed the same labelling pattern as those placed in labelled nutrient after 1 day.

Plants transferred to labelled nutrient at 9 and 12 days were the only treatments, aside from controls, to have some unlabelled syncytia. In these same roots were syncytia with moderately heavy label, so that often one

FIG. 2. Histological preparations of cotton roots inoculated with *Meloidogyne incognita*. Numbers indicate the relative diameter ( $\mu\text{m}$ ) of the white circles in which the letters and numbers appear. A and C-J are autoradiographs. A) Treatment 1, 48 hours after inoculation. Note two unlabelled nuclei in same cell. B) Syncytium 48 hours after inoculation with four synchronized nuclei in anaphase. C) Treatment 2. Label in syncytial nuclei 72 hours after inoculation. D) Treatment 3, 6 days. Late second stage larva and its syncytium showing label localized around periphery of syncytial nuclei. E) Treatment 3, 6 days. Variation in amount of label of nuclei in adjoining syncytia. F) Treatment 5, 12 days. Longitudinal section through entire gall with young adult female, lightly labelled syncytial nuclei, and heavily labelled cortical nuclei. G-H) Treatment 6, 15 days. Adult females associated with labelled syncytium. I-J) Treatment 10. Label applied 9 days after inoculation, with incorporation of label into one syncytium of complex (I) and differential labelling of syncytia (J). Arrows with wavy tails =  $^3\text{H}$ -labelled nuclei and arrows with straight tails, unlabelled nuclei. p = parasite.

giant cell of a set was conspicuously more labelled than the others (Fig. 2-H, I, J).

In all roots harvested at 15 days (including controls) nematodes and giant cells were at the same stage of development, and there did not appear to be any effect of radioactive solutions on either parasite or host.

## DISCUSSION

Previous reports (3, 4, 6, 9, 11) implicating cell wall dissolution during the formative stages of giant cell ontogeny have been confirmed by our results. The occurrence of unlabelled, binucleate giant cells (Fig. 2-A) 48 h after inoculation, and 24 h after continuous incubation in solutions containing  $^3\text{H}$ -thymidine, supports the contention that these cells have arisen by a process of amalgamation of adjacent cells. Nuclei which remained unlabelled 24 h after incubation in  $^3\text{H}$ -thymidine, may also be explained by DNA replication prior to thymidine treatment, but mitosis occurring during the treatment.

However, in contrast with Rubenstein and Owens (13), we observed synchronous mitoses in giant cells within 48 h of inoculation (Fig. 2-B). Since the nuclei resulting from these divisions contained  $^3\text{H}$ -thymidine (Fig. 2-C), it would appear that elements of both cell-wall dissolution and endomitoses play an important role in the formation of multinucleate giant cells.

Synthesis of DNA within giant cells was a continuous process for the length of the experiment. Roots transferred to the radioactive nutrient solution at 9 days had a few giant cells with unlabelled nuclei. In these roots, and those transferred at 12 days, giant cells with all nuclei unlabelled were found, as well as those with all nuclei labelled. Synthesis of DNA by nuclei within individual giant cells appears to be synchronized to the point that all nuclei appear uniformly labelled and that this label was acquired in 3 days or less.

There is no clear explanation why giant cell nuclei are less heavily labelled than surrounding parenchyma cells. Several authors have observed the localization of chromatin in bundles on the periphery of the nuclear membrane [chromatinbildung (4, 8) or Feulgen bodies (1)]; and, according to Christie (4), Nemeč proposed the loss of chromatin into the surrounding cytoplasm. Bird (1) supported and extended this hypothesis through observation of

appropriate electron micrographs. Localization of chromatin in lobes on the nuclear periphery has also been shown in electron micrographs by Paulsen and Webster (12). Loss of chromatin from nuclei would help explain the relatively light label we observed in nuclei continuously exposed to  $^3\text{H}$ -thymidine. The high background label in giant cell cytoplasm may further indicate lost chromatin, or simply show that thymidine accumulates along with many other compounds. Another explanation would be that since giant cell nuclei associated with adult nematodes are enlarged, the labelled DNA is spread over a larger volume to give the impression of less label per unit area.

Except for abnormally enlarged nucleoli, nuclei in giant cells associated with second-stage larvae appeared normal. Mitotic figures also appeared normal. Many other workers (4, 7, 8, 14) have observed mitoses during the first few days of giant-cell development without mentioning any abnormal appearance or behavior of these nuclei. Therefore, a multinucleate condition would appear to develop, in part, from normal mitosis of a number of ordinary nuclei.

In the present study, nuclei in giant cells associated with adults were found to be about twice the diameter of nuclei in younger syncytia. Nuclei of giant cells of cotton do not reach the monstrous proportions of those in some hosts such as sweet potato where the volumes of giant cell nuclei are  $\times 100$  normal (9). Dropkin and Nelson (6) also report diameters of nuclei in fully developed Type IV giant cells which are twice those of nuclei in less-well-developed giant cells. Similar size increases, at the time of completion of molts, have been shown by Bird (2) and Paulsen and Webster (12).

Several authors have observed synchronous mitoses in older giant cells associated with late larval stages or adults. These divisions usually occur in one giant cell of the group, and are often associated with an increase in nuclear size and the appearance of polyploidy. Owens and Specht (11) observed synchronous mitoses of 20 or more nuclei with  $4n$  or more chromosomes. Krusberg and Nielson (9) found simultaneous mitoses at 7 days and Bird (2) found them at 9 and 13 days after inoculation. Dropkin (5) reported seeing synchronous mitoses of polyploid nuclei, some with more than 100 chromosomes, in *Vicia faba*. In the present study, giant cells

with relatively heavily-labelled nuclei were found in roots placed in  $^3\text{H}$ -thymidine 9 days or more after inoculation. The heavy label was limited to nuclei of one giant cell of the group, and probably resulted from synchronous DNA synthesis within the cell, since all nuclei within the cell appeared to be evenly labelled.

We propose, therefore, that the multinucleate giant cells in roots of host plants infected with *M. incognita* arise through a combination of cell wall dissolution, resulting in a coalescence of adjacent cells, and a series of endomitotic divisions. The different patterns of  $^3\text{H}$ -thymidine labelling in syncytial nuclei formed late in giant cell development may reflect an increase in ploidy at a time when the nematode is completing its final molt.

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