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Dynamics of the Nuclear Complement of Giant Cells Induced by *Meloidogyne incognita*

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Abstract: The total numbers of nuclei in giant cells induced by *Meloidogyne incognita* in pea, lettuce, tomato, and broad bean were determined. Mature giant cells from pea had the most nuclei per giant cell with a mean of 59 ± 23, lettuce had the fewest with 26 ± 16, and tomato and broad bean were intermediate. The rate of increase in numbers of nuclei for all plant species was greatest during the first 7 days after inoculation. No mitotic activity was observed in giant cells associated with adult nematodes. Number of nuclei per giant cell doubled each day during the period of greatest mitotic activity, but number of total chromosomes per giant cell increased 20-fold per day at the same time. The hypothesis is presented that factor(s) responsible for the polyploid, multinucleate condition characteristic of giant cells may be different from factor(s) responsible for aneuploid numbers of chromosome per nucleus or for nuclear aberrations such as the presence of linked nuclei.

Key words: broad bean, chromosomes, giant cell, *Lactuca sativa,* lettuce, *Lycopersicon esculentum, Meloidogyne incognita,* nematode, nucleus, *Pisum sativum,* root-knot nematode, tomato, *Vicia faba.*

The transfer cell-like giant cells induced by root-knot nematodes (*Meloidogyne* spp.) are characteristically multinucleate, with multiple nucleoli per nucleus (3,6). Each individual nucleus of the giant cell is usually polyploid with respect to chromosome number. In *Vicia faba,* the polyploid giant cell nuclei appear to be euploid with a regular progression of chromosomes per nucleus (7) or to have at least an even number of chromosomes per nucleus (2). In *Pisum sativum,* the giant cell nuclei are aneuploid (15). Additionally, the DNA content of giant cell nuclei is elevated as compared to that of unaffected root-tip cells, with the content 6- to 16-fold higher in giant cell nuclei (15). There was no apparent relationship, however, between the ploidy level and total DNA per nucleus. Although it has been suggested that the elevated DNA concentrations of giant cell nuclei might result from specific gene amplification (11,15), evaluation of the relative copy number of four specific gene sequences provided no evidence for this hypothesis (16). Numerous studies revealed synchronous mitotic activity in giant cells, with all nuclei of the same giant cell dividing at the same time (2,7,11), especially during the early stages of giant cell development. These reports further stated that mitosis is rarely observed in mature giant cells (those associated with adult nematodes).

Whereas there is a substantial body of knowledge on the altered state of individual nuclei of giant cells, few data are available on the total number of nuclei per giant cell or the dynamics of nuclear number per giant cell. Mature giant cells of *Ipomea batatas* induced by *M. incognita* reportedly have more than 50 nuclei/giant cell (12), *Glycine max* had as many as 150 nuclei/giant cell (5), and *V. faba* infected with *M. javanica* had as many as 48 nuclei/giant cell (2). The objectives of this study were to examine further the total number of nuclei and the rate at which they increase in individual giant cells induced by *M. incognita.*

MATERIALS AND METHODS

The isolate of *M. incognita* race 3 (#82-2) used for these studies was originally isolated from cotton (*Gossypium hirsutum*) and maintained on tomato (*Lycopersicon esculentum*), and it was the same isolate used in previous studies on giant cell nuclei (15,16). Freshly hatched, second-stage juveniles (J2) were used as inoculum and were obtained from excised root-cultures (8). Infected roots were treated with 0.5% NaOCl (9) to dissolve the egg masses, and eggs collected from these cultures were

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placed at room temperature for 48 hours to hatch in chambers constructed from polyvinylchloride pipe and 20-μm pore nylon screen (14).

Fifty freshly hatched J2 per seedling were used to inoculate tomato (Rutgers), pea (*Pisum sativum* 'Little Marvel'), lettuce (*Lactuca sativa* 'Grand Rapids'), and broad bean (*Vicia faba*). Seed of each plant species were germinated and inoculated in "ragdolls" (4) when radicles were 4 to 5 cm long for tomato and lettuce or 8 to 10 cm long for pea and broad bean. Inoculated seedlings were incubated in a controlled environment at 26 C with a 14-hour day. Developing galls were excised from the inoculated roots of pea at 4, 5, 7, 10, 15, and 21 days after inoculation (DAI) and fixed in cold absolute ethanol: acetic acid (70/30, v/v). These times were selected because 4 DAI was the earliest time giant cells could be distinguished, 10 DAI was just prior to molting of the juveniles, 15 DAI was immediately after molting, and at 21 DAI egg production by adult females was observed. Developing galls were collected from tomato, lettuce, and broad bean at 7, 16, and 21 DAI and placed in cold fixative. Samples were processed for microscopy after 24 to 72 hours in the fixative at 4 C, or they were transferred to 70% ethanol and stored for up to 6 months at 4 C before examination.

Gall tissue samples were stained with the Feulgen staining procedure (13), placed in a drop of acetocarmine stain (10) on a glass slide, and dissected to remove the developing giant cells. The large size and enlarged, darkly stained nuclei of giant cells readily distinguished them from surrounding cells. The acid hydrolysis step of Feulgen staining softened the tissue sufficiently to allow dissection of the giant cells with a fine needle. Isolated giant cells were transferred to acetocarmine (10) on a clean slide for microscopic observation with bright field optics.

The experiment to determine rate of increase of nuclei per giant cell in pea was conducted twice, and the data were combined for analysis. A combined total of 26 to 40 giant cells collected from a total of five different pea plants were examined at each sampling time. In studies with tomato, lettuce, and broad bean, a total of 10 giant cells from a total of three different plants were examined at each sampling time and were compared to data from the second experiment with pea. Nuclear count data were subjected to analysis of variance to determine effects of time and host. A regression model of number of nuclei per giant cell in pea against DAI was obtained from the curve-fitting function of Cricket Graph version 1.3 (Cricket Software, Malvern, PA).

Results

Pea seedlings infected with *M. incognita* had a mean of 13 ± 7 nuclei per giant cell at 4 DAI, with a maximum of 34 nuclei/giant cell at 4 DAI. Juveniles associated with giant cells at this time were unswollen. Numbers of nuclei per giant cell continued to increase rapidly until 10 DAI, with a further small increase in number of nuclei being observed at 15 DAI (Fig. 1). Second-

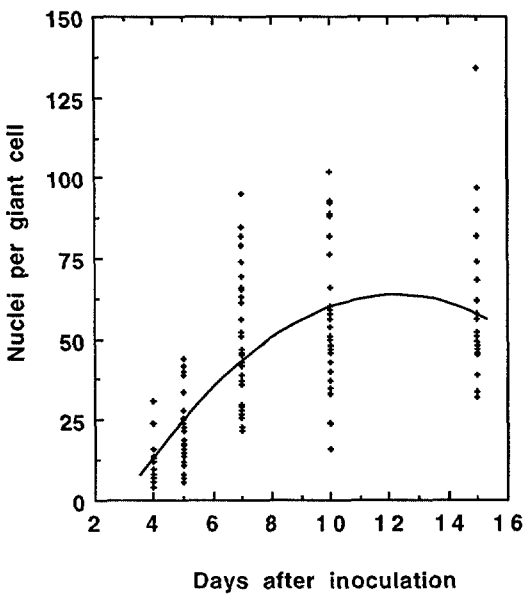

FIG. 1. Increase in number of nuclei per giant cell over time in pea (*Pisum sativum*) infected by *Meloidogyne incognita*. Each point is the number of nuclei from a single giant cell.

stage juveniles associated with giant cells at 5, 7, and 10 DAI cell had mean body diameters of 23 ± 11 μm, 77 ± 25 μm, and 98 ± 19 μm, respectively. At 15 DAI, most of the giant cells were associated with immature adult female nematodes. By 21 DAI, the nuclei were highly aggregated, and accurate counts were not possible. At this time most nematodes were adult females that had commenced egg production. The highest number of nuclei found in any single giant cell was 134, with numerous giant cells having more than 75 nuclei each. Number of nuclei per giant cell at feeding sites induced by a single juvenile were highly variable at all sampling times, with coefficients of variation ranging from 28 to 83%. The relationship between number of nuclei per giant cell (Y) and DAI (X) was best described by the quadratic model $Y = -47.7 + 18.1X - 0.7X^2$ ($R^2 = 0.51$, $n = 170$).

Numbers of nuclei per giant cell in tomato, lettuce, and broad bean at 7 DAI were variable among the hosts, and all were lower than that observed with pea ($P = 0.05$) (Fig. 2). As with pea, the mean numbers of nuclei per giant cell for these three hosts exhibited a further increase at 16 DAI, with an apparent decline at 21 DAI for tomato. The number of nuclei per giant cell in broad bean was unchanged from 16 DAI to 21 DAI. Because nuclei in giant cells of lettuce were highly aggregated at 21 DAI, counts were not attempted.

Giant cells of the different species differed also in shape. Giant cells in tomato and lettuce were globular with a generally smooth surface (Fig. 3A), whereas giant cells from *V. faba* were highly irregular in shape, with long, finger-like projections (Fig. 3C). Giant cells from pea were intermediate in shape, i.e., they varied from being smooth surfaced to having small, finger-like projections (Fig. 3B).

Various degrees of synchrony of mitosis were observed in giant cells of all four plant species. In 60 giant cells from pea observed at 4 and 5 DAI, 14 were in mitosis, and mitosis was synchronous in all 14

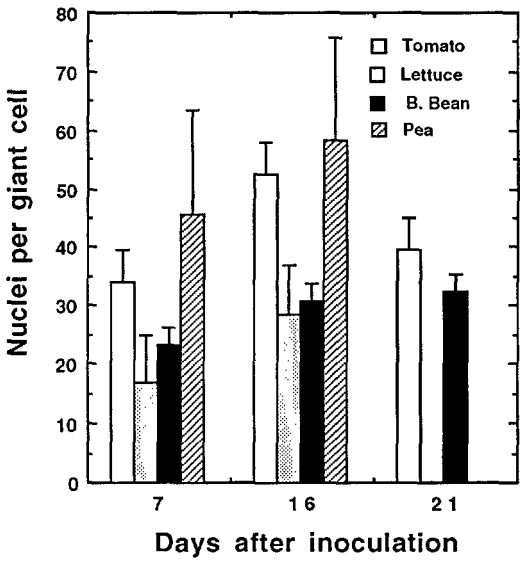

FIG. 2. Comparison of numbers of nuclei per giant cell from pea (*Pisum sativum*), tomato (*Lycopersicon esculentum*), lettuce (*Lactuca sativa*), and broad bean (*Vicia faba*) infected with *Meloidogyne incognita*. Values are means of 10 individual giant cells at each sample date; bars indicate standard error of the means. Data for pea and lettuce at 21 days after inoculation are not shown because nuclei were clumped and could not be accurately counted.

giant cells (Fig. 4A). At 7 DAI complete synchrony was observed in 6 of 35 giant cells from pea; and partial synchrony was observed in 2 of 35 giant cells, where mitosis was confined to nuclei located at one end of the giant cell with remaining nuclei in interphase (Fig. 4B). Partial synchrony was observed in 1 of 39 giant cells from pea at 10 DAI, and complete synchrony was not detected. Mitosis was not detected in giant cells from any host species at 15 or 21 DAI. In addition, in many giant cells two or three apparently distinct nuclei were linked by one or more strands of chromatin.

DISCUSSION

The observations herein confirm and extend our knowledge of the multinucleate condition of giant cells induced in susceptible hosts by the feeding activities of *Meloidogyne* spp. The observed increase in

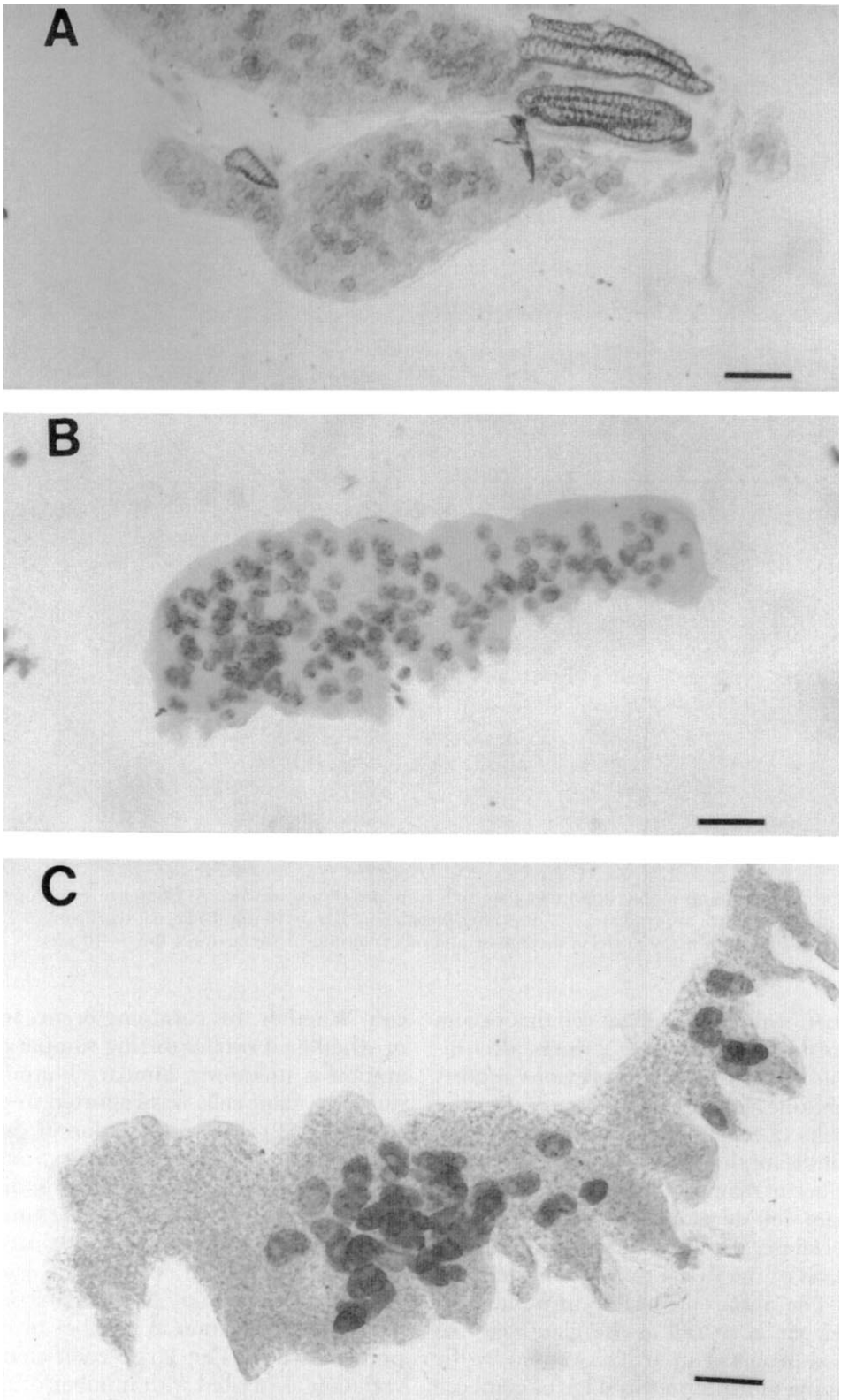

FIG. 3. Giant cells at 8 days after inoculation; note the differences in overall shape, especially the prominent finger-like projections on the giant cells from broad bean (*Vicia faba*). A) Tomato (*Lycopersicon esculentum*). B) Pea (*Pisum sativum*). C) Broad bean. Bar = 100 μm.

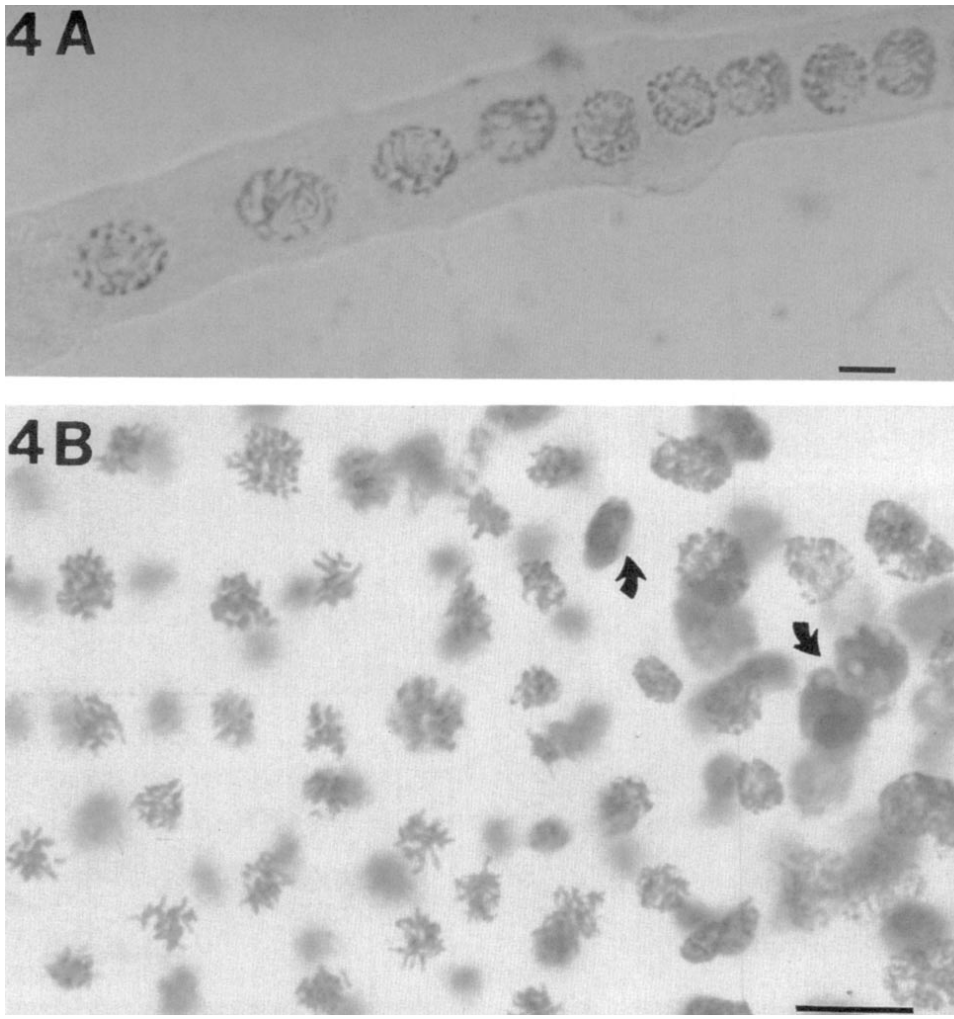

FIG. 4. Synchronous mitotic activity in giant cells from pea (*Pisum sativum*). A) Complete synchrony of 16 nuclei (9 nuclei shown at prophase) at 4 days after inoculation. Bar = 10 μm. B) Partial synchrony at 10 days after inoculation with many nuclei at metaphase and others at interphase (arrows). Bar = 10 μm.

number of nuclei per giant cell that occurs primarily during the first 2 weeks after infection is consistent with previous reports that mitotic activity seldom occurs after the J2 molts (2,7,11). The data from pea further indicate that high levels of mitotic activity occur mainly during the first 7 days of giant cell development, as the number of nuclei per giant cell nearly doubles during each of the first 4 to 6 days after infection. The apparent decline in number of nuclei per giant cell as the giant cell matures is probably an artifact caused by the clumping of nuclei at this stage of giant cell development, making accurate count difficult. Whether this clumping occurs in situ or whether it occurs during sample preparation is unknown. Similar clumping of nuclei in giant cells was reported in other studies (5,12) involving fixation of gall tissues for paraffin embedding.

There was a wide range in body diameter of juveniles at each sampling time for pea, indicating that nematode infection and development on pea was not synchronous; this asynchrony likely is one source of observed variation in number of nuclei per giant cell. The large coefficients of variation associated with number of nuclei per giant cell at feeding sites induced by

one nematode indicated that the observed variation in nuclear number is not caused solely by asynchrony of nematode development but is a real phenomenon.

In a previous study, the individual nuclei of giant cells from pea were characterized as being both polyploid and aneuploid, with chromosome numbers as high as 112/nucleus (15). Over half of the giant cell nuclei in that study had greater than 4n chromosome numbers at 8 DAI, with a mean of 37 ± 19 chromosomes/nucleus. Thus, an average mature giant cell in pea with approximately 60 nuclei (as revealed in the present study) would have nearly 2,220 chromosomes per giant cell, a value 158 times greater than the 2n = 14 of unaffected root cells. Therefore, while numbers of nuclei in giant cells double during each of the first few days of giant cell development, the total numbers of chromosomes are increasing at nearly a 20-fold daily rate. This extremely high level of mitotic activity in the giant cells may be responsible for the numerous aberrations observed in these nuclei. Developing giant cells may be unable to produce sufficient tubulin for the needed numbers of spindle fibers or the attachment of spindle fibers to the kinetochore (1) may be faulty, thus giving rise to incomplete or unequal separation of chromosomes during anaphase.

Numerous questions remain to be answered with respect to the nuclear complement of giant cells, including the identification of the factor(s) responsible for elevated mitotic activity. Because the mitotic activity ceases before development of adult females, there is likely involvement of some specific factor(s) beyond general stimulation of cellular activity resulting from the nematode's feeding activities. The facts that all giant cell nuclei are polyploid and that only some have aneuploid chromosome numbers or nuclei linked by strands of chromatin suggest that the factors responsible for polyploidy differ from those responsible for aneuploidy and linked nuclei.

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