

Cellular Responses of Resistant and Susceptible Soybean Genotypes Infected with *Meloidogyne arenaria* Races 1 and 2¹

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Abstract: The cellular responses induced by *Meloidogyne arenaria* races 1 and 2 in three soybean genotypes, susceptible CNS, resistant Jackson, and resistant PI 200538, were examined by light microscopy 20 days after inoculation. Differences in giant-cell development were greater between races than among the soybean genotypes. *M. arenaria* race 1 stimulated small, poorly formed giant-cells in contrast with *M. arenaria* race 2, which induced well-developed, thick-walled, multinucleate giant-cells. The number of nuclei per giant-cell was variable, but fewer nuclei were usually present in giant-cells induced by race 1 (mean 16 nuclei) than in giant-cells induced by race 2 (mean 41 nuclei). Differences observed in giant-cell development were related to differences in growth and maturation of *M. arenaria* races 1 and 2 and host suitability of the soybean genotypes.

Key words: histopathology, giant-cell, *Glycine max*, *Meloidogyne arenaria*, nuclei, pathogenicity, plant introduction, resistance, root-knot nematode, soybean

Root-knot nematodes, *Meloidogyne* spp., are widely distributed pathogens that establish a complex and long-lasting parasitic relationship with more than 2,000 plant species (23). To complete their life cycles, infective second-stage juveniles (J2) penetrate roots of susceptible hosts behind the root cap (8) and migrate intercellularly to the developing protoxylem in the vascular cylinder (27). Initial feeding activities by J2 induce localized modification of host cells to form a feeding site consisting of several multinucleate giant-cells (7,14). These highly specialized feeding sites function as permanent sources of nutrients for further development and reproduction of the parasite (8,24).

Giant-cells are larger-than-normal cells with multiple nuclei, thickened walls with extensive ingrowths, and dense cytoplasm with increased numbers of organelles (2,13). These feeding sites are highly active metabolically and function as transfer

cells, serving as a metabolic sink for host photosynthates that are consumed by the nematode (3,18). The morphological changes involved in the formation and development of giant-cells have been thoroughly studied. The multinucleate state arises from repeated karyokinesis uncoupled from cytokinesis (7,15), but the DNA content of the nuclei increases nonsystematically on a per-nucleus basis (26). Stylet secretions, originating from the nematode's esophageal glands, injected into the cytoplasm of the protoxylem cells are putatively responsible for regulating giant-cell initiation and development (9,12). Other nematode secretions, presumably from the dorsal gland, form feeding tubes, which are structures that are thought to facilitate withdrawal of nutrients by the feeding parasite (11).

Induction and maintenance of giant-cells are possibly separate phenomena mediated by different developmental stages of the parasite (4). After feeding for about 10-12 days in roots of susceptible plants, the J2 ceases feeding and molts three times over a 48-hour period. Subsequently, the female feeds and grows considerably larger than the giant-cells. The increases in nutrient demands and giant-cell growth correspond to egg production by the female (13). After completion of its life cycle, the female nematode dies and the giant-cells degenerate (1).

Received for Publication 1 December 1995.

¹This research was supported by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations, grants provided by the Georgia Agricultural Commodity Commission for Soybeans, and the Government of Brazil, through the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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Microplot (20) and greenhouse (21) studies indicate that races 1 and 2 of *Meloidogyne arenaria* (Neal) Chitwood are capable of reproducing on susceptible (CNS) and, to a lesser extent, on resistant (Jackson, PI 230977, and PI 200538) soybean, *Glycine max* (L.) Merr. However, race 1 developed slower and produced 4–15% as many eggs per root system as race 2 on all genotypes. The present study was conducted to compare the cellular responses of roots of CNS, Jackson, and PI 200538 infected with races 1 and 2 of *M. arenaria*.

MATERIALS AND METHODS

Three soybean genotypes from Maturity Group VII, *M. arenaria* susceptible CNS, and resistant Jackson and resistant PI 200538, were used in this study. Two isolates of *M. arenaria*, representing host races 1 (GA-7, Georgia isolate) (19) and 2 (Govan, South Carolina isolate) (5), were increased on tomato cv. Rutgers (*Lycopersicon esculentum* L.), and eggs were collected using the NaOCl (0.5%) method (10). Inoculum consisted of J2 freshly hatched from eggs during 48 hours, after discarding J2 emerging during the first 24 hours.

Four seeds of each soybean genotype were planted in 474-cm³ styrofoam cups filled with 420 cm³ soil mix (80% sand, 12% silt, 8% clay) previously fumigated with methyl bromide. Seedlings were thinned to one plant per cup after 5 days, and 7 days later were inoculated with a 4-ml suspension of 2,000 J2 of the appropriate nematode isolate per cup. After 48 hours the root systems were washed with tap water to limit penetration to a 48-hour period and the seedlings were transplanted into styrofoam cups containing the same type of soil. Plants were grown under greenhouse conditions (temperature range 21–35°C) with supplemental light from 400-watt multi-vapor phosphor-coated lamps to provide a 16-hour photoperiod.

In preliminary studies, developing galls containing a single nematode were excised from roots at 5 and 10 days after inocula-

tion for each genotype × race combination and processed for histology. No consistent differences in cellular responses were observed among the genotype × race combinations at these two time intervals. Therefore, in the present study, six galls with a single nematode were excised from roots of each genotype × race combination 20 days after inoculation and processed for histology and giant-cell isolation. The 20 days after inoculation corresponded with the greatest differences in development of races 1 and 2 on these soybean genotypes (21).

The galls were fixed overnight in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) at 4°C. After three changes of buffer, galls were post-fixed for 2 hours at 4°C in 1% OsO₄ in the phosphate buffer, and then washed in distilled water and incubated overnight in uranyl acetate (0.25 gm/50 ml). Galls were washed in distilled water, dehydrated in an ethanol series to acetone, and infiltrated with Spurr's resin. Specimens were embedded using 60 × 15 mm Lux Contour Permanox disposable tissue culture dishes and polymerized in an oven at 70°C. At least six embedded root segments for each genotype × race combination were excised and mounted on specimen stubs to yield longitudinal or cross sections of giant-cells. Serial sections were cut 1 μm thick, fixed on glass slides, stained with 1% toluidine blue, and covered with a coverglass affixed with Permount. The serial sections were examined with a compound microscope, and longitudinal and cross sections of giant-cells at their maximum size for each genotype × race combination were photographed.

To determine the number of nuclei per giant-cell, excised galls were fixed in cold absolute ethanol-glacial acetic acid (3:1, v/v) for 48 hours at 4°C and transferred to 70% ethanol for storage at 4°C before examination. Galls were hydrolyzed in 5 N HCl at 25°C for 40 minutes, rinsed in distilled water, and stained for 2 hours in Schiff's reagent (22). After two changes of sulfite water (22), galls were rinsed in dis-

tilled water, placed in a drop of acetocarmine stain (25) on a glass slide, and dissected with a fine needle with the aid of a stereomicroscope. Twenty giant-cells for each genotype \times race combination were collected, mounted in acetocarmine on a glass slide, and the number of nuclei was determined with the aid of a compound microscope.

RESULTS

CNS: The root galls induced by *M. arenaria* race 1 were small and contained poorly developed giant-cells and nematodes (Fig. 1A,B). The giant-cells were small with cytoplasm containing dark inclusions (Fig. 1A). Occasionally some small giant-cells associated with race 1 lacked the dark inclusions (Fig. 1B). The poorly developed giant-cells did not greatly displace the vascular tissue and were associated with limited hyperplasia and hypertrophy of surrounding host cells.

In contrast, large galls and typical giant-cells were induced by *M. arenaria* race 2 in CNS roots (Fig. 1C,D). These giant-cells were large, multinucleate, contained dense and granular cytoplasm, and had markedly thick cell walls (Fig. 1C). The thickness of the cell wall varied, but wall thickening was evident around the entire giant-cell wall. The nuclei of the giant-cells were hypertrophied and contained prominent nucleoli. The number of giant-cells that could be observed near the head of the nematode ranged from 5 to 8. Cross sections through this area revealed that the giant-cells frequently displaced most of the xylem tissue in the vascular cylinder and were surrounded by extensive hyperplastic host tissue (Fig. 1D).

Jackson: Most of the feeding sites induced by *M. arenaria* race 1 contained only a couple of irregularly shaped multinucleate giant-cells. These small giant-cells contained cytoplasm with dark inclusions (Fig. 2A,B). Cross sections through this area indicated these feeding sites occupied a restricted area of the vascular cylinder and were surrounded by limited hyperplastic

and hypertrophied tissue (Fig. 2B). Restricted nematode development was associated with this type of feeding site.

Feeding sites induced by race 2, on the other hand, usually consisted of a group of 5 to 7 large giant-cells. The giant-cells were multinucleate, had slightly thickened walls, and the cytoplasm, which contained many small vacuoles, was moderately dense (Fig. 2C,D). The large giant-cells displaced the xylem tissue in the vascular cylinder and were surrounded by extensive hyperplastic and hypertrophied tissue (Fig. 2D).

PI 200538: Poorly developed giant-cells were associated with feeding sites in galls induced by *M. arenaria* race 1. Frequently, giant-cells in various developmental stages were present in the same feeding site (Fig. 3A,B).

Race 2 induced large giant-cells that usually filled most of the vascular cylinder. The cytoplasm of these multinucleate giant-cells was dense and contained many small vacuoles (Fig. 3C,D).

Nuclei per giant-cell: The number of nuclei per giant-cell in galls on roots of CNS, Jackson, and PI 200538 at 20 days after inoculation varied within and among the genotypes, but differences were greatest between giant-cells induced by the two races of *M. arenaria* (Fig. 4). The number of nuclei in giant-cells induced in all genotypes ranged from 16 to 64 for race 2 and 8 to 34 for race 1.

DISCUSSION

The differences in development and reproduction of races 1 and 2 (21) on CNS, Jackson, and PI 200538 were related to differences in the development and, presumably, function of giant-cells the pathogens induce in roots of the soybean genotypes. Feeding sites established by race 2 in all genotypes contained large giant-cells capable of supporting the development of J2 to females by 20 days after inoculation, as previously reported (21). However, the giant-cells induced by race 2 in the resistant genotypes contained many small vacuoles and had less thickened cell walls

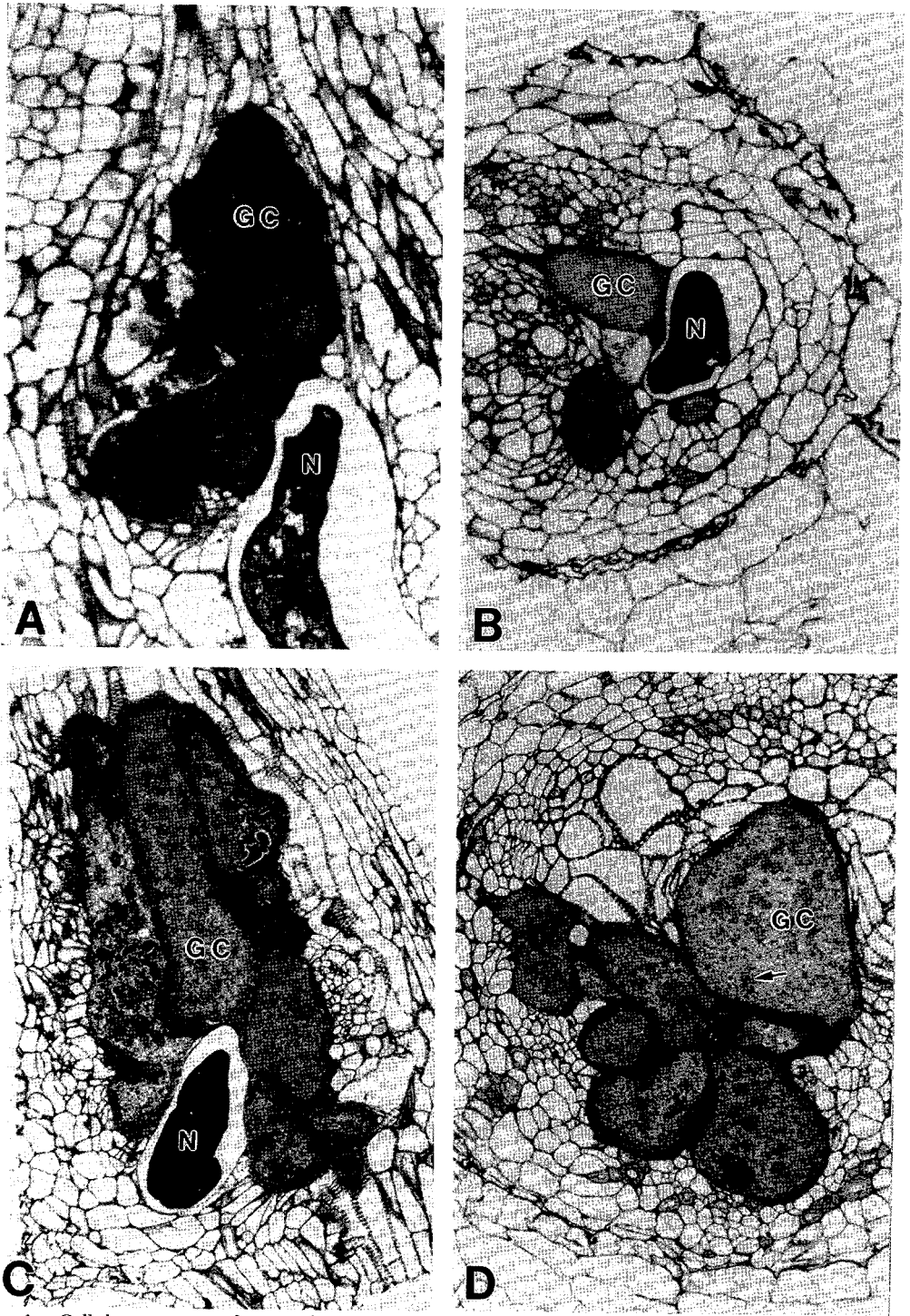


FIG. 1. Cellular response of roots of CNS soybean to infection by *Meloidogyne arenaria* 20 days after inoculation. A,B Race 1: A) Longitudinal section of giant-cells with dark cytoplasm ($\times 319$). B) Cross section of feeding site with small giant-cells with cytoplasm of varying density ($\times 234$). C,D Race 2: C) Longitudinal section of large multinucleate giant-cells with thickened cell walls ($\times 183$). D) Cross section of well developed giant-cells in the vascular cylinder ($\times 192$). GC = giant-cell; N = nematode; arrow = nucleus.

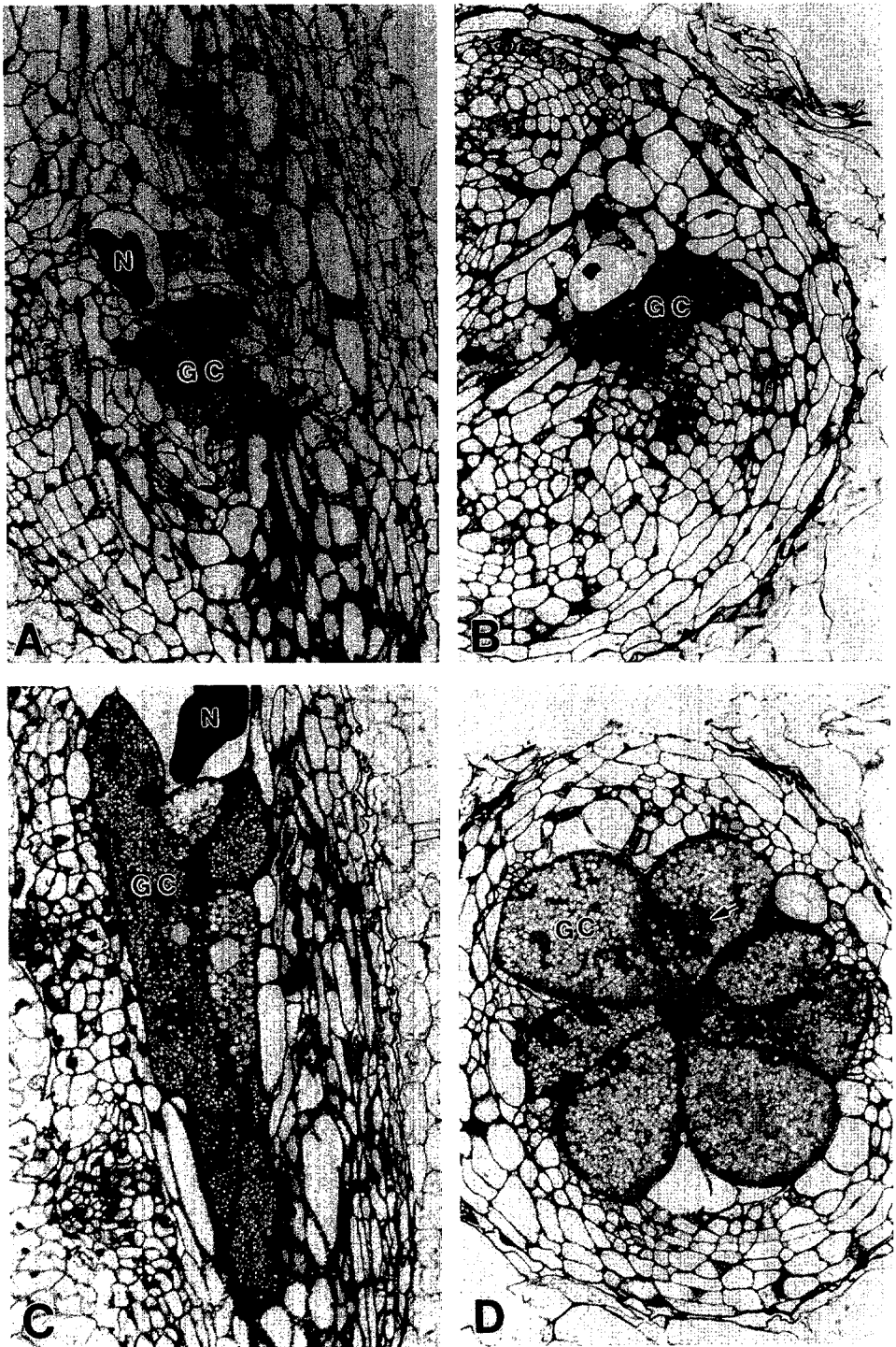


FIG. 2. Cellular response of roots of Jackson soybean to infection by *Meloidogyne arenaria* 20 days after inoculation. A,B) Race 1: A) Longitudinal section of poorly developed giant-cells with dark cytoplasm ($\times 235$). B) Cross section of feeding site with small giant-cells with dark cytoplasm ($\times 270$). C,D) Race 2: C) Longitudinal section of highly vacuolated giant-cells ($\times 205$). D) Cross section of well-developed giant-cells surrounded by hyperplastic and hypertrophied tissue ($\times 296$). GC = giant-cell; N = nematode; arrow = nucleus.

FIG. 3. Cellular response of roots of PI 200538 soybean to *Meloidogyne arenaria* 20 days after inoculation. A,B) Race 1: A) Longitudinal section of feeding site with giant-cells varying in development and cytoplasm density ($\times 247$). B) Cross section of poorly developed giant-cells with dark cytoplasm ($\times 131$). C,D) Race 2: C) Longitudinal section of highly vacuolated giant-cells ($\times 163$). D) Cross section of multinucleate giant-cells ($\times 221$). GC = giant-cell; N = nematode; arrow = nucleus.

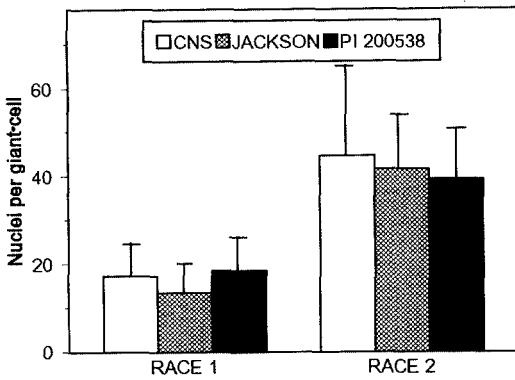


FIG. 4. Numbers of nuclei per giant-cell induced by *Meloidogyne arenaria* races 1 and 2 in galled roots of CNS, Jackson, and PI 200538 soybean. Values are means from 20 individual giant-cells at 20 days after inoculation. Bars indicate standard error of the mean.

compared to giant-cells induced by this race in roots of CNS. These differences in giant-cells may indicate variation in the nutrient status of the feeding sites that could account for the lower fecundity of race 2 females on the resistant genotypes compared to CNS (21).

Giant-cells induced by race 1 in the three soybean genotypes were similar to giant-cells described as type 2 (small giant-cells with abundant cell inclusions of various forms) by Dropkin and Nelson (6) in soybean infected with *M. arenaria*. Type 2 giant-cells were always associated with poorly developed nematodes that produced few eggs. The small size of the feeding sites induced by race 1 in our study should affect the quantity and (or) quality of nutrients the pathogen can obtain, resulting in the decreased rate of development and reproductive potential of race 1 on these soybean genotypes (20,21). The number of nuclei per giant-cell in different susceptible host species is variable (25). In this study, the number of nuclei per giant cell differed only between the two races with the lowest number of nuclei in giant-cells associated with race 1. This restricted number of nuclei probably is related to the limited giant-cell development associated with race 1.

PI 200538 has a higher level of resistance to *M. arenaria* race 2 than Jackson

(16,20). In addition, recent inheritance studies indicate that different genes condition the resistance to *M. arenaria* in these two soybean genotypes (17). Nevertheless, major differences were not detected in the cellular responses in these two soybean genotypes to either race of *M. arenaria*. Interestingly, necrosis, previously associated with resistance to *M. arenaria arenaria* in some soybean genotypes (6), was not observed in infected roots of Jackson or PI 200538.

LITERATURE CITED

- Bird, A. F. 1962. The inducement of giant cells by *Meloidogyne javanica*. *Nematologica* 8:1-10.
- Bird, A. F. 1974. Plant response to root-knot nematodes. *Annual Review of Phytopathology* 12: 69-85.
- Bird, A. F., and B. R. Loveys. 1915. The incorporation of photosynthates by *Meloidogyne javanica*. *Journal of Nematology* 7:111-113.
- Bird, D. M. 1992. Mechanisms of *Meloidogyne*-host interactions. Pp. 51-59 in F. J. Gommers and P. W. T. Maas, eds. *Nematology from molecule to ecosystem*. Wageningen, The Netherlands: European Society of Nematologists.
- Carpenter, A. S., and S. A. Lewis. 1991. Aggressiveness and reproduction of four *Meloidogyne arenaria* populations on soybean. *Journal of Nematology* 32:232-238.
- Dropkin, V. H., and P. E. Nelson. 1960. The histopathology of root-knot nematode infections in soybeans. *Phytopathology* 50:442-447.
- Huang, C. S. 1985. Formation, anatomy, and physiology of giant cells induced by root-knot nematodes. Pp. 155-164 in J. N. Sasser and C. C. Carter, eds. *An advanced treatise on Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.
- Hussey, R. S. 1985. Host-parasite relationship and associated physiological changes. Pp. 143-153 in J. N. Sasser and C. C. Carter, eds. *An advanced treatise on Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.
- Hussey, R. S. 1989. Disease-inducing secretions of plant-parasitic nematodes. *Annual Review of Phytopathology* 27:123-141.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter* 57:1025-1028.
- Hussey, R. S., and C. W. Mims. 1991. Ultrastructure of feeding tubes formed in giant cells induced in plants by the root-knot nematode *Meloidogyne incognita*. *Protoplasma* 162:99-107.
- Hussey, R. S., E. L. Davis, and C. Ray. 1994. *Meloidogyne* stylet secretions. Pp. 233-249 in F. Lamberti, D. De Giorgi, and D. McK. Bird, eds. *Advances*

in molecular plant nematology. New York: Plenum Press.

13. Jones, M. G. K. 1981. Host cell responses to endoparasitic nematode attack: Structure and function of giant cells and syncytia. *Annals of Applied Biology* 97:353-372.

14. Jones, M. G. K., and D. H. Northcote. 1972. Multinucleate transfer cells induced in coleus roots by the root-knot nematode, *Meloidogyne arenaria*. *Protoplasma* 75:381-395.

15. Jones, M. G. K. and H. L. Payne. 1978. Early stages of nematode-induced giant cell formation in roots of *Impatiens balsamina*. *Journal of Nematology* 10:70-84.

16. Luzzi, B. M., H. R. Boerma, and R. S. Hussey. 1987. Resistance to three species of root-knot nematode in soybean. *Crop Science* 27:258-262.

17. Luzzi, B. M., H. R. Boerma, and R. S. Hussey. 1995. Inheritance of resistance to the peanut root-knot nematode in soybean. *Crop Science* 35:50-53.

18. McClure, M. A. 1977. *Meloidogyne incognita*: A metabolic sink. *Journal of Nematology* 9:88-90.

19. Noe, J. P. 1992. Variability among populations of *Meloidogyne arenaria*. *Journal of Nematology* 24:404-414.

20. Pedrosa, E. M. R., R. S. Hussey, and H. R. Boerma. 1994. Response of resistant soybean plant introductions to *Meloidogyne arenaria* races 1 and 2. *Journal of Nematology* 26:182-187.

21. Pedrosa, E. M. R., R. S. Hussey, and H. R. Boerma. 1996. Penetration and post-infectious development and reproduction of *Meloidogyne arenaria* races 1 and 2 on susceptible and resistant soybean genotypes. *Journal of Nematology* (in press).

22. Price, H. J., K. Bachmann, K. L. Chambers, and J. Riggs. Detection of intraspecific variation in nuclear DNA content in *Microseris douglasii*. *Botanical Gazette* 141:195-198.

23. Sasser, J. N. 1980. Root-knot nematodes: A global menace to crop production. *Plant Disease* 64:36-41.

24. Sijmons, P. C., H. J. Atkinson, and U. Wyss. 1994. Parasitic strategies of root-knot nematodes and associate host cell responses. *Annual Review of Phytopathology* 32:235-259.

25. Starr, J. L. 1993. Dynamics of nuclear complement of giant cells induced by *Meloidogyne incognita*. *Journal of Nematology* 25:416-421.

26. Wiggers, R. J., J. L. Starr, and H. J. Price. 1990. DNA content and variation in chromosome number in plant cells affected by the parasitic nematodes *Meloidogyne incognita* and *M. arenaria*. *Phytopathology* 80:1391-1395.

27. Wyss, U., F. M. W. Grundler, and A. Munch. 1992. The parasitic behaviour of second-stage of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. *Nematologica* 38:98-111.