Description of the host-parasite relationship of *Meloidogyne christiei* with *Quercus laevis*

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SUMMARY

In Florida, *Meloidogyne christiei* is a widespread root parasite of the turkey oak *(Quercus laevis)* forming nodulelike galls, mainly by hyperplasia. Motile, vermiform juveniles infect lateral root tips at or near the apex. Upon penetration, nematodes appear to migrate through cortical parenchyma in a spiral manner to the phloem where feeding sites are initiated. Feeding sites are comprised of giant cells (hypertrophied phloem parenchyma) which contain granular cytoplasm and many unusual nuclei. Initially, the enlarged ovoid spherical multinucleolate nuclei have smooth envelopes. These nuclei elongate, aggregate, and their envelopes become lobed with age. Giant cell cytoplasm commonly aggregates along the thickened walls which contain numerous pits. Sclerenchyma cells form where galls protrude from the root. Vascular tissue within galls appears disorganized. Young galls containing nematodes had one or two females, one or two males, a gelatinous matrix, and an average of 185 eggs and 52 juveniles. Matrix, eggs, and juveniles are located in a spiral duct which connects the female posterior to the rhizosphere. Duct development is initiated during nematode penetration and is associated with considerable cellular destruction. We hypothesize that the gelatinous matrix elicits the formation of periderm where cortical parenchyma cells had lined the duct. The periderm produces a single layer of suberized cork cells proximal to the female which thickens to one to six layers near the rhizoplane.

RÉSUMÉ

Description des relations hôte-parasite entre Meloidogyne christiei *et* Quercus laevis

En Floride, *Meloidogyne christiei* est un parasite courant du chêne de Turquie *(Quercus laevis)* sur les racines duquel il forme des galles nodulaires, essentiellement par hyperplasie. Les juvéniles vermiformes, mobiles, infestent les racines latérales à leur extrémité ou près de celle-ci. Après pénétration, le nématode migre à travers le parenchyme corticale, suivant une voie en spirale vers le phloême où les sites nutritionnels sont induits. Ces sites sont constitués de cellules géantes (parenchyme du phloême hypertrophié) contenant un cytoplasme granuleux et de nombreux noyaux aberrants. Au début, les noyaux, ovoïdes ou sphériques, multinucléolés, ont une enveloppe lisse; ils s'allongent ensuite, se groupent et leur enveloppe devient lobée. Le cytoplasme des cellules géantes se rassemble en général le long des parois, épaissies et pourvues de nombreuses perforations. Des cellules sclérenchymatiques sont formées là où les galles font saillie sur les racines. A l'intérieur des galles, les tissus vasculaires apparaissent désorganisés. Les jeunes galles renferment une moyenne de 185 œufs et 52 juvéniles, et une substance gélatineuse. Cette substance ainsi que les œufs et les juvéniles sont situés dans un conduit spiralé qui connecte la partie postérieure de la femelle à la rhizosphère. La réalisation de ce conduit commence lors de la pénétration du nématode et est associée à une destruction cellulaire notable. Nous supposons que la substance gélatineuse induit la formation du périderme constitué de cellules du parenchyme cortical bordant le conduit. Ce périderme comprend une seule couche de cellules subérisées au voisinage de la femelle et s'épaissit (cinq à six couches cellulaires) près de la rhizoplane.

Meloidogyne christiei Golden & Kaplan, 1986, was recently described following its detection in galls on roots of turkey oak, *Quercus laevis* Walt., in Florida. The unusual appearance of the galls and the monospecific host range of this nematode (Golden & Kaplan, 1986) suggest that this nematode-plant relationship is highly specialized. This paper includes descriptions of *i)* the feeding site of *M. christiei, ii)* spiral ducts which occur within the galls, and *iii)* the numbers of eggs, juveniles, and adults occuring within the galls.

Materials and methods*

Galled roots of *Quercus laevis* were collected from Sanlando Park, Altamonte Springs, Florida, from July through January. Roots were gently rinsed free of soil

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and debris with tap water and individual galls were excised. Single galls placed in BPI watch glasses containing water were teased apart under a stereoscopic microscope. A 1 mm square grid was then placed under each watch glass and the total numbers of eggs, juveniles, females, and males were counted. Gall diameters were measured using a calibrated eyepiece micrometer.

Galled tissue was fixed in FAA, transferred to 10 % formalin in 95 % ethanol (v/v), dehydrated with a tert-butyl alcohol-ethanol series, and embedded in high melting point Paraplast® at 62°. Sections 10 µm thick were cut and stained with different combinations of safranin, fast green, hemalum, hematoxalin, eosin, erytrosin B, orange G, methyl green, crystal violet and methylene blue. Select sections were photographed with a Zeiss Photomicroscope II.

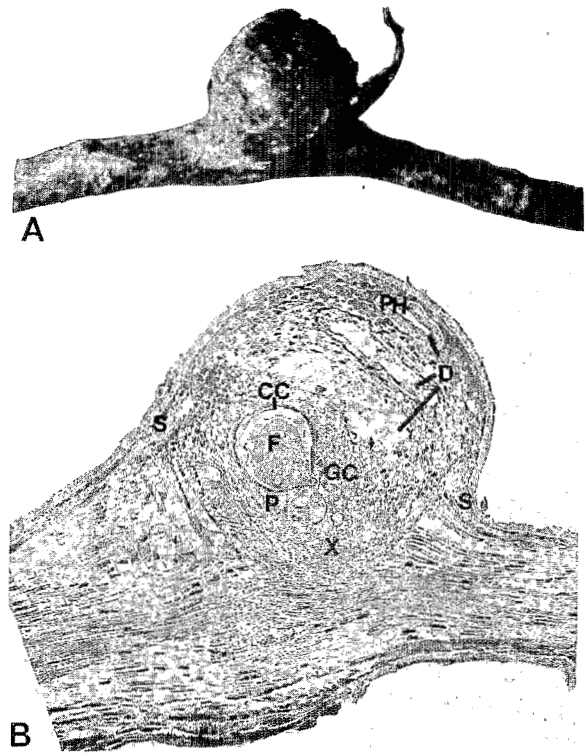

Fig. 1. *Meloidogyne christiei.* A : Orientation of galls on root of turkey oak. B : Longisection of gall. Note the general orientation of the nematode, feeding site, root and modified structures associated with parasitism (F = female; E = egg; G = giant cell; S = sclerenchyma cells; X = xylem; P = phloem; D = duct; CC = compressed cortical cells; PH = phellem).

Results

Galls commonly occurred individually or in clusters (two to five) on one side of the root (Fig. 1 A); swelling or distortion of the entire root circumference was not apparent. There were usually no branch roots at or near the galls. Galls isolated from roots were 2.4 (± 0.31) mm in diameter and were typically spheroid and rigid. Young

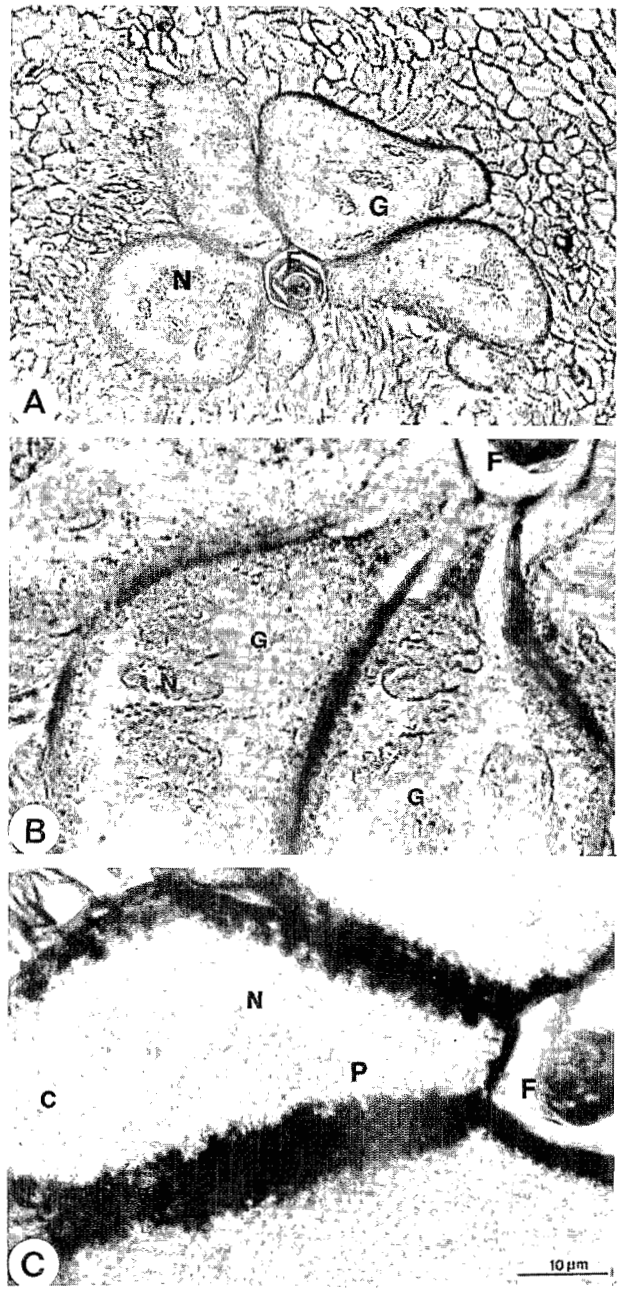

Fig. 2. *Meloidogyne christiei.* Feeding sites of adult females. A : General orientation of female, giant cells, and nuclei; B : Hypertrophied giant cells exhibiting aggregation of elongated, lobed nuclei; C : Hypertrophied giant cells stained with hemalum to differentiate the thickened walls and presence of pits (W + wall; P = pit; F = female; C = cytoplasm; N = nuclei; G = giant cells).

galls appeared tan or orange, and became dark brown and hardened with age. Of 90 randomly selected galls, only 32 contained eggs and/or juveniles. The number of eggs ranged from 6 to 443 ($\bar{x}$ = 185.2; s = 123.2) and the number of juveniles ranged from 0 to 244 ($\bar{x}$ = 52; s = 123.2). Galls usually contained one female, seven galls contained two females, and females could not be identified in 29 galls. Nineteen galls had one male, thirteen galls contained two males, and males were not identified in 58 galls. Eggs were oviposited in a gelatinous matrix. Relatively little gelatinous matrix was observed on the gall surface.

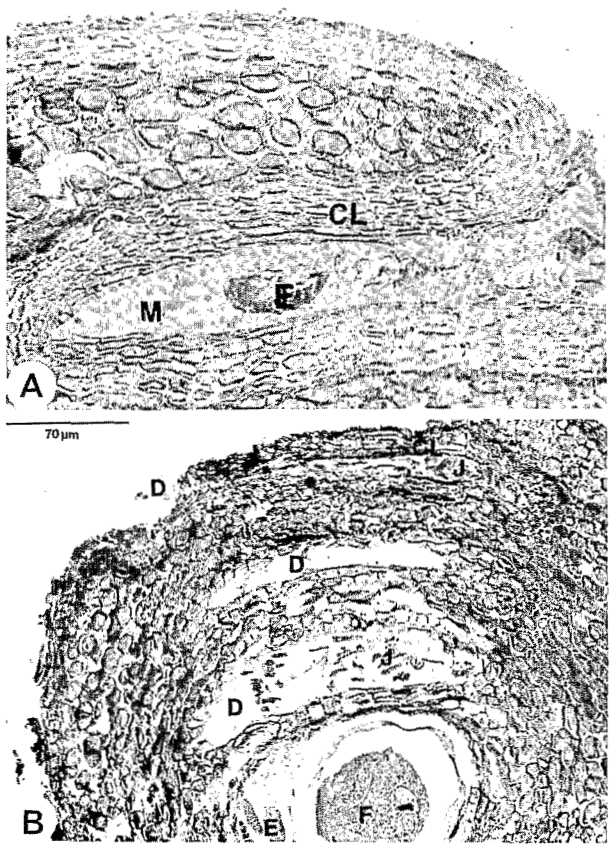

Fig. 3. *Meloidogyne christiei.* A. Opening of duct to the rhizosphere. Ducts were lined with several layers of cork cells and were filled with gelatinous matrix, juveniles, and males; B : Detail of duct containing eggs, juveniles, and matrix. Note the enhanced thickening of cork layer lining the duct as it approaches the rhizoplane (M = matrix; F = female; E = egg; J = juvenile; D = duct; CL = cork layer).

Staining sections with safranin and fast green proved most useful for distinguishing tissues. Sections revealed that both hypertrophy and hyperplasia of cortical cells and hyperplasia with incomplete elongation of xylem cells were largely responsible for the spheroid appearance of galls (Fig. 1 B). Sclerenchyma cells formed in the root cortex where galls protruded from the root surface. Staining (safranin) of cortical parenchyma within the galls intensified toward the gall surface. Vascular tissue within the galls appeared disorganized; xylem cells within galls were centripetally oriented, and extended from the giant cells toward the root axis. These xylem cells were numerous and did not elongate completely, but did exhibit secondary wall thickenings. Feeding sites were comprised of giant cells that surrounded the heads of females within the phloem (Fig. 2 A). Young giant cells contained granular cytoplasm, numerous small vacuoles, and many enlarged, ovoid-spherical multinucleolate nuclei. The nuclei elongated, aggregated, and their envelopes became lobed with age (Figs 2 A, B). Dense giant cell cytoplasm commonly lined differentially stained thick walls, which appeared to contain numerous pits (Fig. 2 C). Females occurred within cavities bounded by compressed parenchyma cells (Fig. 3 A). These cavities were continuous with a spiral duct which opened to the rhizoplane. Proximal to the female, the duct was lined only with compressed cortical parenchyma cells. However, distal to the female, the duct wall was comprised of four to six layers of cork cells (Fig. 3 B) which were continuous with the outer wall (exodermis) of the gall. Female enlargement appeared to compress cortical cells proximal to the cuticle (Fig. 1 A). Male development was similar to that described for other root-knot nematode species (Fig. 4 A).

Discussion

In Florida, *M. christiei* is a widespread root parasite of turkey oak, *Q. laevis.* Growth of trees parasitized by *M. christiei* did not appear to be impaired; however, we did not conduct field trials to assess the impact of *M. christiei* on the growth of turkey oak. The impact of these galls on root or tree physiology has not been assessed. Visible effects on roots are limited to laterally located spheroidal galls on otherwise normal roots.

Penetration was associated with considerable cellular destruction (Fig. 3 B). Nematodes migrated through cortical parenchyma in a spiral manner to the procambium possibly to lateral root initials. A spiral duct within each gall appeared to develop from the route taken during nematode penetration. Feeding sites were then stimulated in young, actively-dividing cells which resulted in swelling of the host tissue (Huang, 1985; Hussey, 1985). In contrast to galls on roots of pin oak (*Quercus palustris* Muenchh.) associated with *Meloidogyne querciana* Golden, 1986, and root galls on a wide array of plants associated with many other root-knot nematode species; galls associated with *M. christiei* on turkey oak roots were limited to the tissues immediately surrounding the nematode and its feeding site (Huang, 1985; Hussey, 1986).

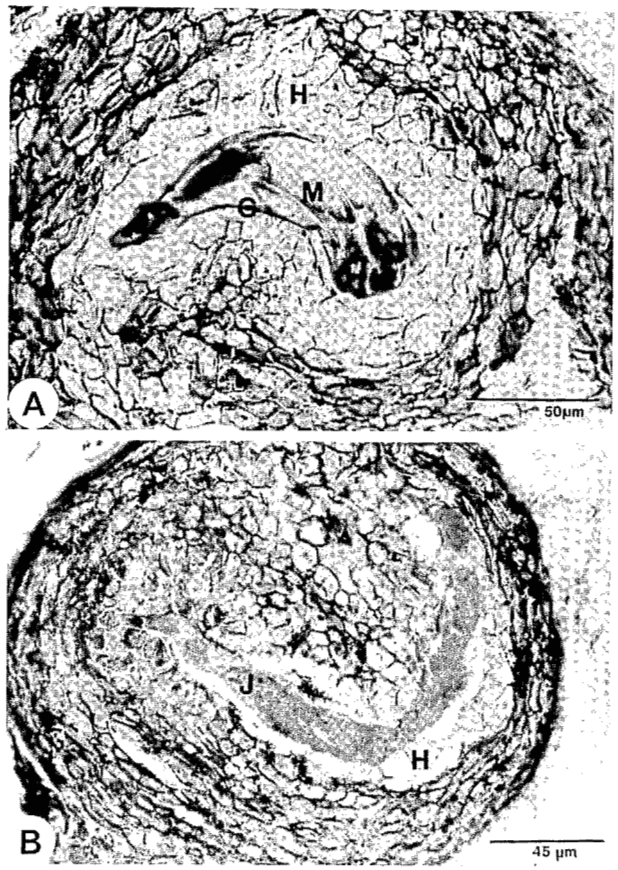

Fig. 4. *Meloidogyne christiei.* A : Adult male prior to emergence from fourth stage cuticle. (M = male, C = fourth stage cuticle, H = hypertrophied cells); B : Cross section of root tip infected by juvenile (M = male; C = fourth stage cuticle; H = hypertrophied cells; J = juvenile).

The nematode penetration pathway later became filled with hypertrophied parenchyma cells subsequently replaced by a gelatinous matrix produced by the female root-knot nematode. The matrix may possess cellulytic activity as suggested by Orion, Loots and Orion (1987). The gelatinous matrix appeared to elicit the formation of periderm by the cortical parenchyma which had initially lined the penetration pathway (Fig. 3 B). The periderm subsequently produced a single layer of suberized cork cells proximal to the female; the cork cells increased to four to six layers near the rhizoplane (Fig. 3 A). Suberization may be steongly influenced by the rhizosphere environment; oxygen and/or dessication may mediate cork cell formation (Esau, 1953). The matrix bound by the duct wall could be dissected out of the galls as a tubular, coiled, structure (Golden & Kaplan, 1986), rather than a globular mass as is common in other rootknot species.

Ducts associated with *Meloidogyne christiei* parasitism of *Q. laevis* roots are functionally similar to the penetration pathway of *Hylonema ivorense* described in roots of *Turraeanthus africana* by Taylor, Cadet and Luc (1978). Penetration pathways associated with both nematode species contain thickened walls and the entire duct can be dissected from the root. While the functions of the penetration pathways and giant cells are similar, the organization of the walls of the penetration pathways and cells within the feeding sites suggests that the association of *M. christiei* with *Q. laevis* is more highly evolved than that of *H. ivorense* with *T. africana.*

Feeding sites associated with *M. christiei* appear similar to those associated with other *Meloidogyne* species (Huang, 1985); they occur within the stele with the female body extending out into the root cortex, nuclei are highly lobed, irregular, and aggregated within giant cells (Jones & Northcote, 1972; Himmelhoch *et al.,* 1973). As for all compatible host-parasite interactions involving *Meloidogyne* species, the formation of giant cells resultant from repeated mitoses and cell enlargement rather than from cell fusion appears essential to the development of *M. christiei* on *Q. laevis.* Unique aspects of this host-parasite relationship include the nodulelike appearance of the galls, monospecific host range, the organization of canals formed during invasion of the root and which later serve as a juvenile escape route, and the strategic location of sclerenchyma cells at the base of the galls which may strengthen gall attachment to the root. The nematode's morphology (Golden & Kaplan, 1986) is also unique.

In the present study, we did not observe a relationship between gall size and number of juveniles or eggs contained within the gall (r = 0.006). Samples were collected through fall, winter and spring. It is possible that the production of eggs and subsequent juvenile development are seasonal or dependent on some undetermined environmental factor which did not occur during the course of this study. Host reactions which impair nematode development may also account for the relatively high frequency of galls of different sizes which were void of juveniles or eggs.

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REFERENCES

ESAU, K. (1953). *Plant Anatomy.* New York, Wiley & Sons, 735 p.

GOLDEN, M. A. (1986). Descriptions of *Meloidogyne camelliae* n. sp. and *M. querciana* n. sp. (Nematoda : Meloidogynidae), with SEM and host-range observations. *J. Nematol.,* 11 : 175-189.

GOLDEN, M. A., & KAPLAN, D. T. (1986). Description of *Meloidogyne christiei* n. sp. (Nematode : Meloidogynidae) from oak with SEM and host-range observations. *J. Nematol.*, 18 : 533-540.

HIMMELHOCH, S., COHN, E., MORDECHAI, M. & ZUCKERMAN, B. M. (1979). Changes in fine structure of citrus root cells induced by *Tylenchulus semipenetrans. Nematologica,* 25 : 333-335.

HUANG, C. S. (1985). Formation, anatomy and physiology of giant cells induced by root-knot nematodes. In : Sasser, J. N. & Carter, C. C. (Eds). *An Advanced Treatise of* Meloidogyne. *Vol. 1 : Biology and Control,* Raleigh, N. Carolina St. Univ. Graphics : 155-164.

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HUSSEY, R. S. 1985. Host-parasite relationships and associated physiological changes. In : Sasser, J. N. & Carter, C. C. (Eds) *An advanced Treatise of* Meloidogyne. *Vol. 1 : Biology and Control,* Raleigh, N. Carolina St. Univ. Graphics : 143-155.

JONES, M. G. K. & NORTHCOTE, D. H. (1972). Nematode-induced syncytiun — A multinucleate transfer cell. *J. Cell Sci.,* 10 : 789-809.

ORION, D., LOOTS, G. C. & ORION, T. (1987). Cell lysis activity of *Meloidogyne* gelatinous matrix. *Revue Nématol.,* 10 : 463-465.

TAYLOR, D. P., CADET, P. & LUC, M. (1978). An unique host-parasite relationship between *Hylonema ivorense* (Nematoda : Heteroderidae) and the roots of a tropical rainforest tree. *Revue Nématol.,* 1 : 99-108.