Journal of Nematology 44(2):148–152. 2012.

Description of a Unique, Complex Feeding Socket Caused by the Putative Primitive Root-Knot Nematode, *Meloidogyne kikuyensis*

J.D. Eisenback,[1] D.J. Dodge[2]

Abstract: Meloidogyne kikuyensis produces unique galls that form on one side of the root resembling nitrogen-fixing nodules that are produced on legumes in response to infection by *Rhizobium* and related bacteria. The gall caused by this root-knot nematode is made up of a complex feeding socket composed of several giant cells that are ramified with xylem vessels extending perpendicular from the vascular cylinder. The anterior portion of the second-stage juvenile, which develops into an adult, plugs into this unique feeding socket. The socket and the surrounding parenchyma together form a gall that is very different in morphology from those typically caused by other species of root-knot nematodes. Even though *M. kikuyensis* was considered to be a primitive species because of its low chromosome count, the complexity of its feeding site and minor plant damage suggests a more derived systematic position.

Key words: feeding site, gall, giant cell, histology, host-parasitic relationship, morphology, nodule, *Rhizobium*, SEM, sugarcane, systematics.

Meloidogyne kikuyensis de Grisse, 1960 was first described as a parasite of kikuyu grass (*Pennisetum clandestinum* Höchst.) in Kenya (de Grisse, 1960). This species differs from other root-knot nematodes both morphologically and cytologically. It has only 7 very large chromosomes in contrast with much smaller and more numerous chromosomes (13-19 n) of other *Meloidogyne* species (Triantaphyllou, 1985; 1990). For this reason Triantaphyllou suggested that *M. kikuyensis* is a primitive species of the genus. Likewise the morphology of the anterior region of the male also supports the idea that it is a primitive species because the labial disk and six lips remain distinct and separated from each other (Eisenback and Spaull, 1988), whereas these structures of more highly evolved species are fused together (Eisenback and Hunt, 2009).

Species of the genus *Meloidogyne*, the root-knot nematodes, typically cause galls on the roots where a second-stage juvenile (J2) establishes a feeding site and develops into either a male or a sedentary, pear-shaped female with an increased reproductive capacity (Taylor and Sasser, 1978; Caillaud et al., 2008). Development of the gall occurs when the nematode penetrates the root as a J2 and migrates to the differentiating vascular cylinder and initiates a feeding site among the pro-xylem cells. The J2 secretes pharyngeal exudates that initiate transcription factors that cause repeated mitosis without cytokinesis. This results in the formation of giant cells which, in combination with hypertrophy of the surrounding cortical cells, is responsible for galling that occurs around the vascular cylinder of the root (Paulson and Webster, 1970). This process usually occurs around the anterior end of the nematode where the nematode is parallel to the root axis.

The morphology of the gall caused by *M. kikuyensis* is unique from other species as was reported in the original description on kikuyu grass (de Grisse, 1960). *Meloidogyne kikuyensis* forms a gall on one side of the root, perpendicular to the root axis, resembling a nitrogen-fixing nodule that is produced by bacteria on leguminous plants (de Grisse, 1960). This may be why cowpea [*Vigna ungulculata* (L.) Walp.], which forms nitrogen-fixing nodules, may have been mistaken as a host for *M. kikuyensis*. It also causes the same type of gall on sugarcane (Eisenback and Spaull, 1988) and yellow foxtail (*Setaria glauca*, L.) (Pers. Comm., Eisenback). The majority of the *Meloidogyne* species do not differ in gall formation on susceptible plants (Taylor and Sasser, 1978), although *Meloidogyne hapla* Chitwood, 1949 forms a gall that is somewhat diagnostic (Eisenback et al., 1984), other species may induce extremely large galls on some plants, whereas others may not stimulate the formation of galls at all.

The unique gall induced by *M. kikuyensis* and the suggestion that it is a primitive species of the genus prompted an investigation into the morphology of its unique root gall. In order to understand the morphology of this gall, light microscopy, including histological thin sections, and scanning electron microscopy of whole and cut galls were utilized.

Materials and Methods

Tissue collection, fixation, dehydration, infiltration, embedding, sectioning, ribbon mounting, and staining were based techniques described by Daykin and Hussey, 1985. Parasitized roots from infected sugarcane were washed with tap water to remove adhering particles of soil. Root pieces with galls were cut into small sections (1-2 mm long) and fixed for 24 hrs. in 4% glutaraldehyde, buffered in 0.1M sodium cacodylate (pH 7.2). The fixative volume was more than ten times that of the plant material in order to avoid dilution of the fixative with the water that was inside the roots.

Received for publication April 24, 2012.

[1]Professor, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061.

[2]Undergraduate Senior, Department of Biology, Bryan College, Dayton, TN 37321.

The authors would like to thank Dr. Vaughn Spaull of the South African Sugarcane Research Institute, Mount Edgecombe, South Africa for supplying the specimens of *M. kikuyensis*. We would also like to acknowledge the financial support of the American Lumber Standards Committee, Germantown, MD, and the Council of Higher Education for Virginia Equipment Trust Fund.

E-mail: jon@vt.edu

This paper was edited by Nancy Kokalis-Burelle.

Water contained inside the root tissue was gradually removed with a series of tertiary butyl alcohol (TBA), water, and ethanol to avoid plasmolysis. Over a series of 7 steps of at least 15 min each, the TBA concentration was increased to 100%. Galled root tissue was placed in a 1:1 solution of TBA and paraffin oil (mineral oil). After 1 hour or more the roots were transferred to a small Stendor dish that was ¾ full of slightly liquefied paraffin and placed in an oven at a temperature slightly above the melting point of paraffin (56.5°C). The mineral oil solution in which the roots were contained was placed on top of the slightly solidified paraffin, and after 1-3 hours was poured off and replaced with liquid Paraplast® two times.

Metal mold pieces were coated with glycerin, and a small amount of liquid paraffin was poured into the mold. The root tissue was positioned in the mold according to the desired orientation for cutting either longitudinal or cross-sections. More paraffin was poured through the plastic holder attached to the metal tray. The mold was submerged into cold (10°C) water to quickly solidify the wax.

A microtome was used to cut the root tissues embedded in the paraffin mold into thin sections (10 µm thick). These sections made a ribbon that was transferred to a covered container for dust-free storage. The ribbon was transferred to a glass slide for mounting and staining by floating the ribbon in warm (40°C) water and adhered onto the slide using Haupt's adhesive (Johansen, 1940). Johansen's quadruple technique was used to stain these sections according to Daykin and Hussey (1985).

Scanning electron microscopy (SEM) required fixation, dissection, dehydration, Freon 113 infiltration, freeze-drying, gold coating, and imaging. Sequential fixation as described by Eisenback (1984) was utilized. After 24 hrs in fixative, longitudinal and cross-sections of the galls and roots were cut with a razor blade. Root tissues were placed in a BEEM capsule container (Eisenback, 1984) and kept in a Stendor dish for dehydration, infiltration with Freon 113, and freeze-drying.

The material was freeze-dried in a Virtis 6201 3210 freeze-dryer for 30 min. and mounted with double sticky tape onto aluminum stubs for viewing in the SEM. They were coated with approximately 240 Å of gold/paladium with an Emitech SC 7620 sputter coater operating at 20 mA for 90 seconds. Imaging of the specimens was completed in a JEOL Neoscope JCM-5000 operating at 10 kV.

Results

The galls induced by *M. kikuyensis* on sugarcane occur in two forms (Figs. 1-5.): most commonly, one that is smooth (Fig. 1A) and, rarely, another that is covered with numerous root hairs (Fig. 1B). When the gall is very young, it is slightly translucent (Fig. 1C), but gradually

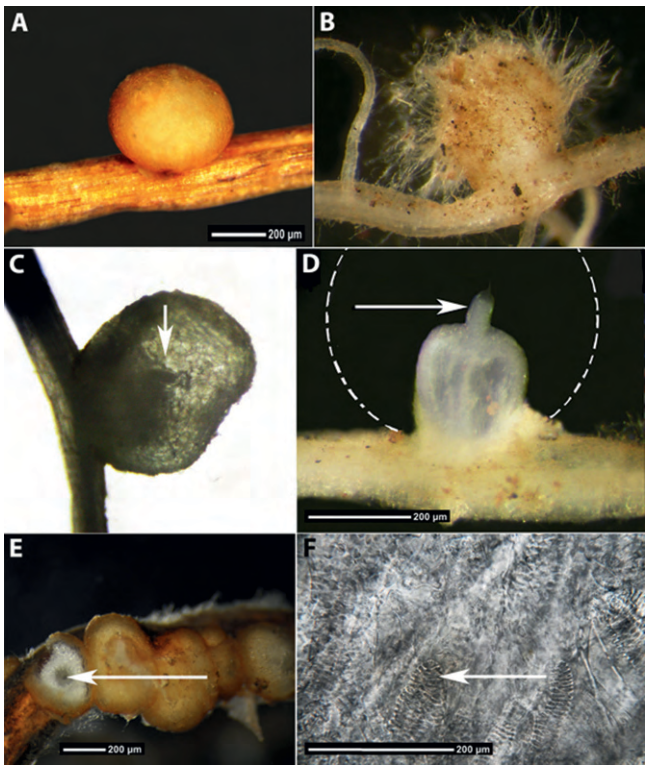

Fig. 1. Light micrographs of galls on sugarcane caused by the root-knot nematode, *Meloidogyne kikuyensis*. A. A solitary, smooth gall that resembles a gall caused by *Rhizobium* spp. on leguminous plants. B. An atypical, solitary gall that is covered with root-hairs. C. A young gall that is small and transluscent, containing a developing second-stage juvenile (arrow). D. A gall that has been dissected to reveal the feeding socket that is formed by giant cells that are ramified with xylem tissues. E. A row of galls with one dissected to show the feeding socket (arrow). F. A feeding socket dissected and mashed between a glass slide and a coverslip showing numerous xylem vessels (arrow) interspersed between several multinucleate giant cells.

becomes more opaque as it increases in size and age. When it is small, the developing J2 can be seen embedded within a more opaque core. This core is called the "feeding socket" because of the way the nematode is "plugged" into it. The outer portion of the gall, composed of mainly parenchyma, can easily be separated from the nematode and the feeding socket to reveal the J2 or mature female. The feeding socket occurs at a 90° angle to the length of the root (Fig. 1D). When the nematode is pulled out of the socket, a hole is clearly visible (Figs. 1E, 3A, 4). Crushing the feeding socket between a coverslip and a glass slide reveals that it is made up of several (4-8) multinucleate giant cells that are ramified with blindly ending vascular elements that are connected at a 90° angle to xylem elements within the vascular cylinder of the root system (Fig. 1F).

The xylem elements of the feeding socket are directed up into the gall and in between the giant cells (Figs. 2-3) where they are clearly demarcated from the remaining part of the gall by several layers of small parenchyma cells (Figs. 2-4). SEM of the gall and cuts through the gall, clearly shows the morphological details of the feeding socket. Cross- and longitudinal sections through a gall

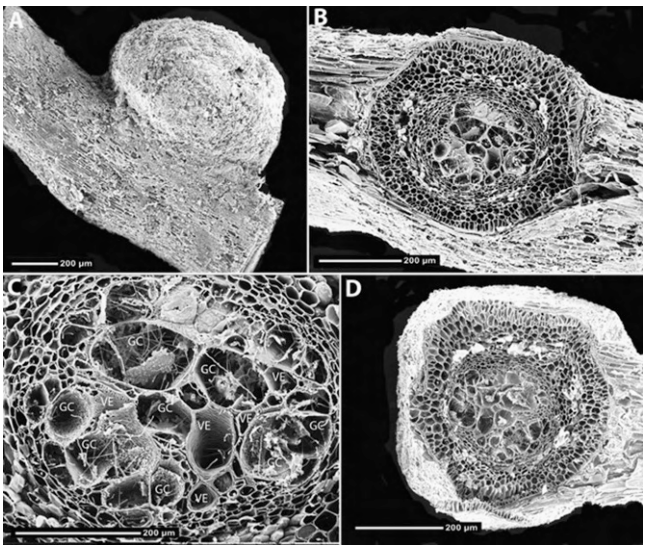

FIG. 2. Scanning electron micrographs of galls on sugarcane caused by *Meloidogyne kikuyensis* de Grisse, 1960. A. Entire gall. B. Root with the gall cut away showing the giant cells and vascular elements of the feeding socket. C. Enlarged view of the giant cells and interspersed vascular elements (GC = giant cell, VE = vascular element). D. Underside of gall cut away from the root showing the feeding socket filled with giant cells and vascular elements.

and attached root shows that the feeding socket is made up of several giant cells that are interspersed with vascular cells. The hole where the nematode head is inserted into the socket (Figs. 3A, 4) after nematode removal is clearly seen when the outer parenchyma cells comprising the outer gall are removed (Fig. 4). Scanning electron micrographs of the exposed feeding socket show the location of insertion for the nematode and the xylem cells that closely surround the head of the feeding nematode (Fig. 4D). Light microscopy (Fig. 4C) shows an enhanced view of the hole left by the nematode in the feeding socket after the gall has been excised. Major differences occur in the morphology and nature of the feeding site of a typical root-knot nematode and the feeding socket formed by *M. kikuyensis* (Fig. 5.).

DISCUSSION

Meloidogyne kikuyensis is unlike other species in the genus because of the type of gall that it causes (de Grisse, 1960). In addition, this species differs dramatically from other *Meloidogyne* species in cytology, physiology, and morphology (de Grisse, 1960; Eisenback & Spaull, 1988; Triantaphyllou, 1990). Typically in the root-knot nematodes, the giant cells are interspersed among the vascular elements of the central cylinder of the root where the body of the nematode remains parallel with xylem cells (Bird 1974; Taylor and Sasser,

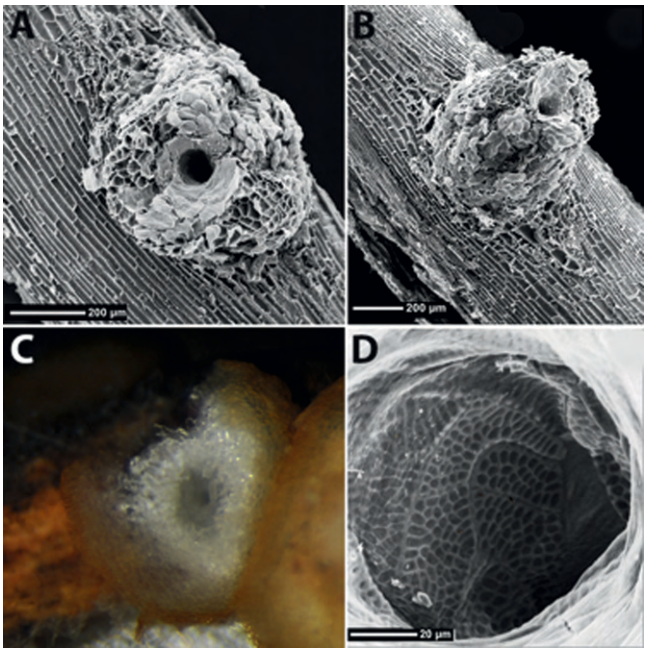

FIG. 3. Scanning electron micrographs (SEM) and light micrograph of a gall caused by *Meloidogyne kikuyensis* de Grisse, 1960 on sugarcane. A. SEM of a gall, cut longitudinally, revealing the feeding socket containing giant cells and vascular tissues surrounded by large parenchyma cells. B. SEM of a gall cut in cross-section revealing the feeding socket and cavity where the female was contained (imaged at same scale as A). C. Light micrograph of a thin-section through the longitudinal plane of a gall showing the giant cells and xylem tissues forming the complex feeding socket (N = nematode). (Arrows demarcate feeding socket.)

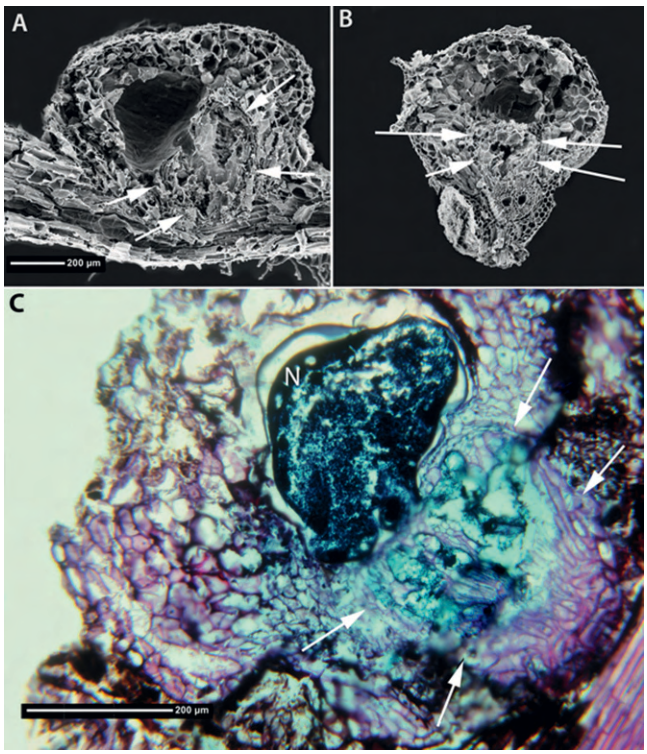

FIG. 4. Scanning electron micrographs (SEM) and light micrograph (LM) of the feeding socket contained within a gall on sugarcane caused by the root-knot nematode, *Meloidogyne kikuyensis* de Grisse, 1960. A-B. SEM of a dissected gall with the parenchyma cells removed to reveal the feeding socket where a second-stage juvenile or adult female feeds. C. LM of a dissected gall showing the feeding socket from which a mature female was removed. (Imaged at same scale as A and B.) D. SEM of the hole within the feeding socket that is lined with vascular tissues.

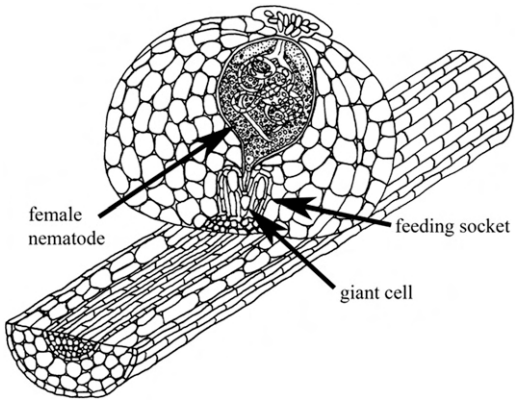

FIG. 5. Diagram of the unique gall with a distinct feeding socket caused by *Meloidogyne kikuyensis* de Grisse, 1960 on sugarcane.

1978; Wergin and Orion, 1982); however, in *M. kikuyensis*, a feeding socket is formed by an extension of the vascular cylinder where it and the body of the nematode are perpendicular to the vascular cylinder. This type of gall production appears to be more complex than the typical giant cell system that is formed in the vascular cylinder (Wergin and Orion, 1982).

The complex morphology of the feeding socket and gall may be responsible for less disruption of the root system in *M. kikuyensis* as compared to the typical root-knot gall. The feeding socket is tapped into the vascular cylinder, but this feeding site does not block the flow of nutrients through the root as is common with the other more typical giant cell systems. This arrangement may be less pathogenic to the plant because *M. kikuyensis* has not been observed causing major economic injury in host plants.

Meloidogyne kikuyensis reproduces by obligatory amphimixis (Triantaphyllou, 1990), which may be one of the reasons why it is difficult to maintain in culture. Therefore, details about penetration by the J2 and the initiation of the feeding site remain unknown. These details may be possible to investigate if a more suitable host can be utilized. Our attempts to culture this nematode on cowpea and kikuyu grass have not been successful; however our inoculum has always been sparse.

The formation of galls caused by *M. kikuyensis* has not been observed directly, but their development may be quite different from the typical root-knot gall because of the orientation of the feeding socket with the vascular cylinder (Bird, 1961; 1974). One possible explanation of gall formation is that the nematode penetrates an emerging lateral root and stimulates giant cell production that inhibits root elongation. However some roots have galls that are so close together that they coalesce into a continuous line of galls. This occurrence is inconsistent with the idea that galls are initiated on emerging roots because lateral roots usually do not form close to each other.

Further investigation is necessary to reveal the details about gall development. Additional studies about the penetration, initiation, and formation of the gall are desirable, but difficult on sugarcane. These studies are necessary to elucidate the unique and complex host-parasite relationship that *M. kikuyensis* has with its host plant. If this species is indeed a primitive species within the genus *Meloidogyne*, more studies may increase our understanding of the evolution of plant-parasitism within the root-knot nematodes. Molecular phylogenetic studies and additional notes about the morphology of this species will be reported in a separate publication.

The gross morphology of the gall caused by *M. kikuyensis* resembles that of nitrogen-fixing nodules caused by *Rhizobium* and related bacteria on leguminous plants. The symbiotic relationship between bacteria and the plant root is initiated by molecules (Nod factors) that are produced by the bacteria (Ardourel et al., 1994). Root-knot nematodes have also been shown to induce similar chemical signals (NemF), leading investigators to conclude that root-knot nematodes have incorporated a portion of the "symbiont-response pathway to enhance their parasitic ability" (Weerasinghe et al., 2005). Perhaps the unique morphology of the feeding socket of *M. kikuyensis* give credence to that suggestion.

LITERATURE CITED

Ardourel, M., Demont, N., Debellé, F. D., Maillet, F., de Billy, F., Promé, J. C., Dénarié, J., and Truchet, G. 1994. *Rhizobium meliloti* lipooligosaccharide nodulation factors: different structural requirements for bacterial entry into target root hair cells and induction of plant symbiotic developmental responses. Plant Cell 6:1357–1374.

Bird, A. F. 1961. The ultrastructure and histochemistry of a nematode-induced giant cell. Journal of Biophysical and Biochemical Cytology 11:701–715.

Bird, A. F. 1974. Plant response to root-knot nematode. Annual Review of Phytopathology 12:69–85.

Caillaud, M. -C., Quentin, G., Dubreuil, M., Perfus-Barbeoch, L., Lecomte, P., de Almeida Engler, J., Abad, P., Rosso, M.- N., and Favery, B. 2008. Root-knot nematodes manipulate plant cell functions during a compatible interaction. Journal of Plant Physiology 165:104–113.

Daykin, M. E., and Hussey, R. S. 1985. Staining and histopathological techniques in Nematology. Pp. 39–47 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An Advanced Treatise on *Meloidogyne*, Volume II. Raleigh: North Carolina State University Graphics.

Eisenback, J. D. 1984. Techniques for preparing nematodes for scanning electron microscopy. Pp. 70–105 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced Treatise on *Meloidogyne*, Vol. 2. Methodology. Raleigh: North Carolina State University Graphics.

Eisenback, J. D., and Hunt, D. 2009. General morphology. Pp. 18–54 *in* R. N. Perry, M. Moens, and J. L. Starr, eds. Root-Knot Nematodes. Wallingford: CAB International.

Eisenback, J. D., and Spaull, V. W. 1988. Additional notes on the morphology of males of *Meloidogyne kikuyensis*. Journal of Nematology 20:633–634.

Eisenback, J. D., Hirschmann, H., Sasser, J. N., and Triantaphyllou, A. C. 1984. A Guide to the Four Most Common Species of Root-Knot Nematodes. A cooperative publication of the Department of Plant Pathology, North Carolina State University and the

United States Agency for International Development. Raleigh: North Carolina State Graphics.

de Grisse, A. 1960. *Meliodogyne kikuyensis* n. sp. a parasite of kikuyu grass (*Pennisetum cladestinum*) in Kenya. Nematologica 5:303–308.

Johansen, D. A. 1940. Plant microtechnique. New York: McGraw-Hill Book Co.

Paulson, R. E., and Webster, J. M. 1970. Giant cell formation in tomato roots caused by *Meloidogyne incognita* and *Meloidogyne hapla* (Nematoda) infection. A light and electron microscope study. Canadian Journal of Botany 48:271–276.

Taylor, A. L., and Sasser, J. N. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne species*). A cooperative publication of the Department of Plant Pathology, North Carolina State University and the United States Agency for International Development. Raleigh: North Carolina State Graphics.

Triantaphyllou, A. C. 1985. Cytogenetics, cyrotaxonomy and phylogeny of root-knot nematodes. Pp. 113–126 *in* J. N. Sasser, and C. C. Carter, eds. An Advanced Treatise on *Meloidogyne,* Vol. l, Biology and Control. Raleigh: North Carolina State University Graphics.

Triantaphyllou, A. C. 1990. Cytogenetic status of *Meloidogyne kikuyensis* in relation to other root knot nematodes. Revue de Nématologie 13:175–180.

Weerasinghe, R. R., Bird, D. M., and Allen, N. S. 2005. Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. Proceedings of the National Academy of Sciences 102:3147–3152.

Wergin, W. P., and Orion, D. 1982. Scanning electron microscope study of the root-knot nematode (*Meloidogyne incognita*) on tomato roots. Journal of Nematology 13:358–367.