Post-infection Development and Morphology of Meloidogyne cruciani[1]

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Abstract: The development and life stages of *Meloidogyne cruciani* on tomato was studied at 28 C. Roots of 2-wk-old 'Rutgers' tomato seedlings were exposed to inoculum for 24 h, rinsed, and the seedlings repotted. No major changes in juvenile development were observed prior to 8 days after inoculation. At 11 days the second-stage juvenile had enlarged considerably. The genital primordium had not yet asumed the V-shape characteristic of developing females, but the presence of rectal glands identified the juveniles as females. At this time (11 days), two additional, previously undescribed esophageal lobes were first observed; they were adjacent to the dorsal and subventral glands. After molting from second to third stage, the stylet cone, shaft, and the lumen of the stylet knobs are shed and remain attached to the second-stage cuticle. The excretory duct of the third-stage juveniles was directed anteriorly from the excretory pore of the second-stage cuticle and appear attached to the body wall of the third-stage juveniles opposite the procorpus. At 19 days after inoculation, the last molt took place. The adult female possessed a new stylet, a large five-gland esophagus, a prominent excretory system ending in a unicellular gland and a fully developed reproductive system. *Key words:* life cycle, root-knot nematode, esophageal glands, excretory system, rectal glands.

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In most genera of nematodes, adult females resemble the juvenile stages in many of their morphological characters. In the genus *Meloidogyne,* the developing juveniles undergo a series of morphological changes and the mature females appear very different from the second-stage juveniles. The former have a saccate pear-shaped body, a large robust stylet, very prominent esophageal glands, and an enlarged metacorpus. The second-stage juveniles are filiform with a small stylet and small esophageal glands.

Before Chitwood (4) revised *Heterodera marioni* into the genus *Meloidogyne* and created five species and one subspecies, the life cycle and morphological studies of *Heterodera marioni,* now an archaic designation of *Meloidogyne,* were conducted by Nagakura (10) and Christie and Cobb (5). Nagakura described three molts taking place within the plant roots and the existance of third and fourth juvenile stages. Christie and Cobb disagreed with Nagakura, stating that there was no third juvenile stage and that the fourth was theoretical.

More recently, Bird (3), Triantaphyllou and Hirschmann (12), and Siddiqui and Taylor (11) studied the morphology and developmental stages of females of *M. javanica, M. incognita,* and *M. nassi,* respectively. Their results confirmed the early studies of Nagakura and reported the presence of third and fourth juvenile stages.

The work reported herein is an attempt to describe the initiation, development, and formation of morphological structures in the developmental stages of *Meloidogyne cruciani* García Martínez, 1982 (7), with emphasis on the esophageal region, excretory system, and genital region.

MATERIALS AND METHODS

The roots of 2-wk-old seedlings of 'Rutgers' tomato (*Lycopersicon esculentum* Mill.), susceptible to *M. cruciani,* were exposed to freshly hatched second-stage juveniles for 24 h. Then the roots were washed and the seedlings transplanted into plastic cups containing 33 cm^3 of sterile white sand. The seedlings were placed at a constant temperature of 28 ± 1 C and fertilized twice a week with a 12-10-20 nutrient solution. Every 24 h, five seedlings were removed and the roots washed, fixed, and stained using De Guiran's (8) method. After fixation, the developmental stages of *M. cruciani* were dissected from the roots and mounted in glycerin on glass slides.

Morphological observations and drawings were made using Nomarski interference contrast optics.

RESULTS

During the first 7–8 days after inocula-

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tion, the postinfective second-stage juvenile underwent few changes but generally decreased in length and increased in diameter when compared with the preinfective stage (Fig. 1A, B). At 7–8 days, the genital primordium in the four-cell stage began to migrate posteriorly and the esophageal glands became shorter in length but larger in diameter. An increase in size was observed in the metacorpus and in the body circumference in the esophageal region (Fig. 1B). The nucleoli and the chromocenters of the esophageal gland nuclei became enlarged. At this point, the rectal glands were not seen, but six irregularly arranged nuclei were present near the anal region.

Eleven days after inoculation, the second-stage juvenile had enlarged considerably in size (Fig. 1C). The esophageal glands continued to increase in width and volume, with the procorpus and metacorpus enlarging and becoming more prominent. For the first time, two small additional esophageal lobes were seen just posterior to the metacorpus. The dorsal esophageal gland had enlarged more than the subventral ones; chromocenters of all were very prominent. The genital primordium, now in the six-cell stage, had enlarged and migrated posteriorly. Rectal glands were visible in this stage. The genital primordium had not developed to the V-shape characteristic of the developing female gonad, but those juveniles which would develop into females could be recognized by the presence of rectal glands.

Fourteen days after inoculation, the procorpus, metacorpus, and esophageal glands enlarged (Fig. 1D). The two small esophageal lobes, first observed 11 days after inoculation, enlarged and their nuclei could be seen. The nucleoli and chromocenters of the other three esophageal glands were prominent. The genital primordium had assumed the V-shape characteristic of the developing female gonad. The genital primordium was in close proximity to the rectal glands but had not attached itself to the body wall.

Sixteen days after inoculation, the second-stage female juveniles showed further enlargement of the esophagus. Their genital primordia had two branches di-

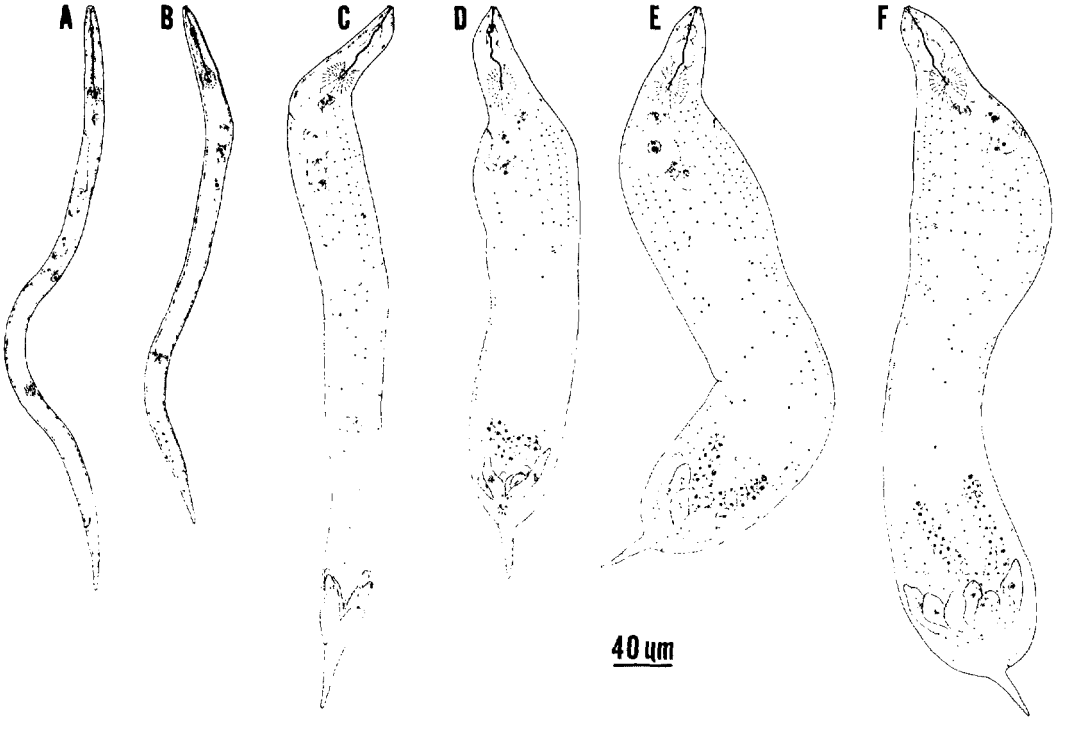

Fig. 1. Second-stage juveniles of *Meloidogyne cruciani*. A) Infective second-stage juvenile. B) Postinfective sexually undifferentiated second-stage juvenile. C–F) Female second-stage juveniles.

rected anteriad which grew in length as the gonad moved towards the anal region and attached to the body wall (Fig. 1E, F). The juveniles possessed six well-developed rectal glands, and a new cuticle was evident posteriorly as the second-stage cuticle began to separate.

The third-stage juveniles (Fig. 2A, B) could be recognized enclosed in the second-stage juvenile cuticle. They did not possess a stylet and their posterior ends were round. The second-stage juvenile stylet remained attached to the old cuticle. During the second molt, the cone and shaft of the stylet and the lumen (inner lining) of the stylet knobs were shed with the second-stage cuticle. The stylet knobs disappeared leaving a void in the anterior part of the body. The esophageal lumen and valve of the metacorpus were still visible but very faint. The esophageal glands lost their chromocenters, but the nucleoli remained promi-

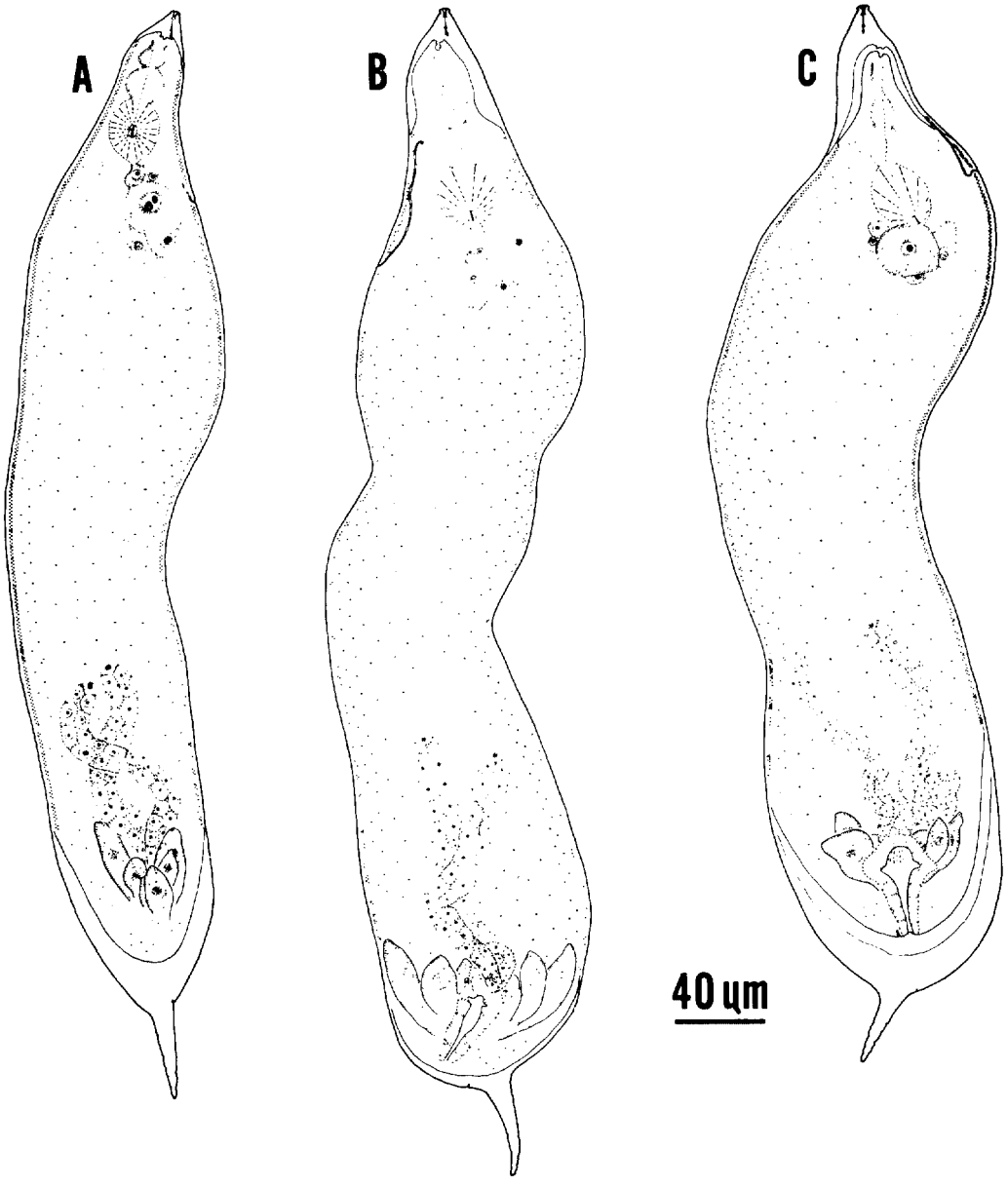

Fig. 2. Third- and fourth-stage juveniles of *Meloidogyne cruciani*. A–B) Third-stage juveniles encased in the second-stage cuticle. C) Fourth-stage juvenile encased in the second- and third-stage cuticles.

nent (Fig. 2B). In the early third-stage juvenile the excretory pore on the second-stage cuticle was located opposite the esophageal glands with a short duct pointing anteriorly (Fig. 2A), while in the late third-stage juvenile the excretory duct extended anteriorly and appeared to penetrate the body opposite the procopus (Fig. 2B). The ovaries of the third-stage juvenile continued to elongate, and the uterus and vagina began to develop.

With the onset of the third molt, the body of the fourth-stage juvenile formed and separated from the third-stage cuticle. The early fourth-stage juvenile (Fig. 2C) differed from the late third-stage juvenile (Fig. 2B) only by being enclosed in both the old second and third juvenile cuticles. No stylet was visible, and a void was seen in this location. The lumen of the esophagus and the valve of the metacorpus were very faint, with the valve now located in the posterior portion of the metacorpus. The excretory duct was similar to that of the third-stage juvenile; it ran anteriorly and penetrated the body opposite the procorpus. The gonads continued to elongate, and the uterus and vagina completed development.

In the early stages of the adult female, shortly after the fourth molt and while still enclosed in the second, third, and fourth juvenile cuticles, the stylet could be seen (Fig. 3A). The esophagus appeared typical of adult females with the procorpus and metacorpus enlarged and prominent. The lumen of the esophagus and valve of the metacorpus were present, appearing faintly at first. Nucleoli remained prominent. On the old cuticle of the second-stage juvenile the excretory pore was posterior to the metacorpus and its duct was leading anteriorly where it appeared to penetrate the female body opposite the procorpus. From here the excretory duct in the adult female body was seen leading posteriorly. The gonads continued to elongate. The uterus, vagina, vulva, and rectal glands were prominent and the perineal pattern was visible. Nineteen days after inoculation, all organs of the adult female were developed, and molting of the second, third, and fourth cuticles occurred simultaneously (Fig. 3B).

Immediately after molting, feeding was resumed and the female enlarged from a sausage shape (Fig. 3C) to a pear shape (Fig. 3D). The stylet was robust and well-developed. The enlarged procorpus had a prominent lumen. The massive metacorpus had a strong well-sclerotized valve. The esophageal glands consisted of five distinct lobes with prominent nuclei and nucleoli. The excretory pore was located opposite the procorpus with the duct leading posteriorly to a unicellular gland. The gonads elongated with one branch extending anteriorly close to the esophageal region. The rectal glands were large with prominent nuclei and nucleoli.

DISCUSSION

The postinfection development of *Meloidogyne cruciani* agrees in general with the studies conducted by Bird (3), Triantaphyllou and Hirschmann (12), and Siddiqui and Taylor (11).

The postinfection second-stage juvenile had a slight decrease in body length when compared to the preinfection juvenile. Bird (3) also found a decrease in size of the infective second-stage juveniles after root penetration; he attributed the decrease in size to the depletion of food reserves used during penetration and migration in the roots. The first noticeable changes that the postinfection juveniles underwent were in body length and in the esophageal region. The esophagus increased in volume, and the body around the esophageal region had a noticeable increase in width. The changes in the esophageal region may have been due to the intense feeding activity of the second-stage juveniles.

Triantaphyllou and Hirschmann (12) reported the shape of the genital primordium (V-shape for females; straight cylindrical shape for males) could be used to differentiate sex in early second-stage juveniles. Triantaphyllou and Hirschmann (12) and Siddiqui and Taylor (11) refer to all those postinfective stages that have not attained the V-shape genital primordium as "developed but sexually undifferentiated" second-stage juveniles. In this study, sex was determined in the second-stage juveniles of *M. cruciani* as early as 11 days after inoculation and before the genital primodium had assumed the V-shape typical of developing

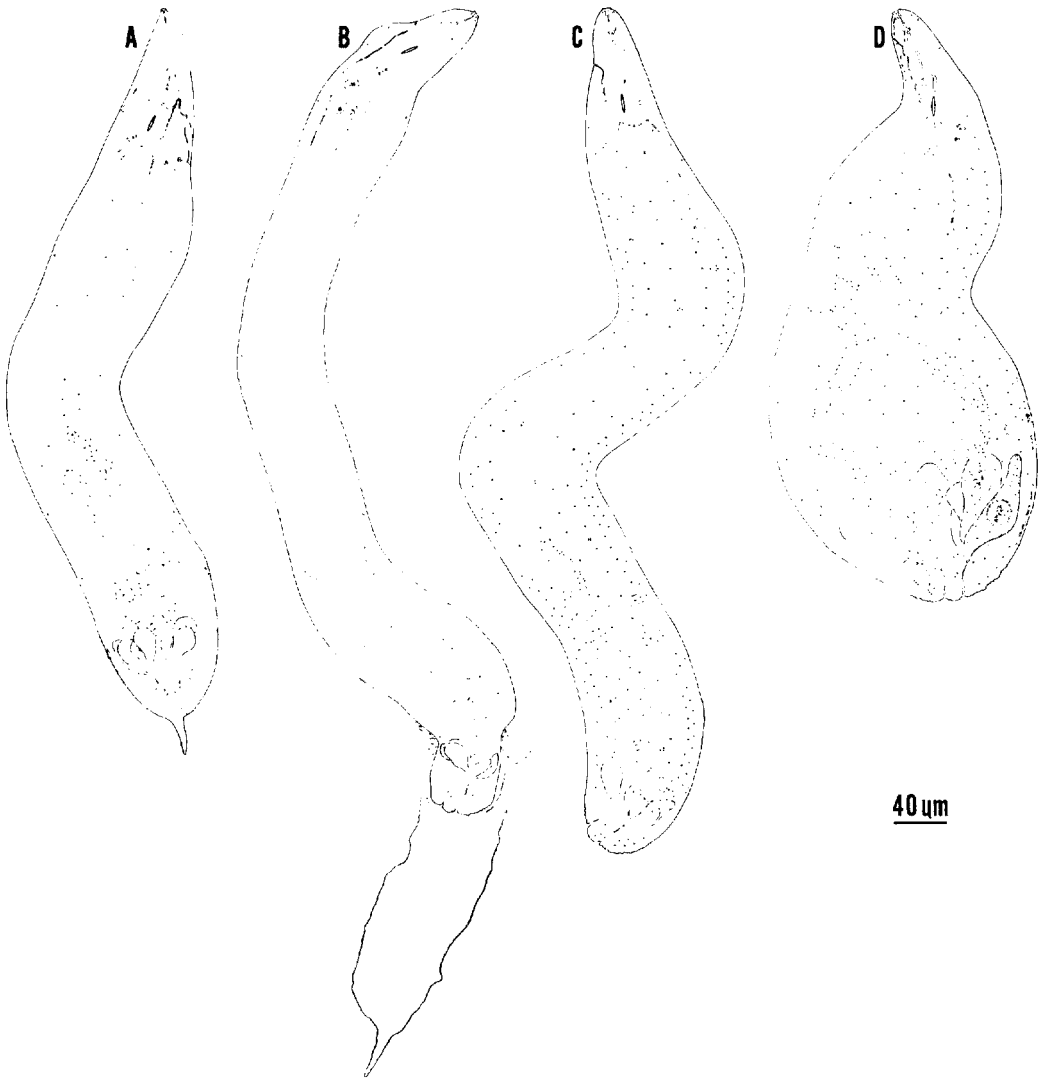

Fig. 3. Adult females of *Meloidogyne cruciani*. A) Early adult female still enclosed in old cuticles of the second-, third-, and fourth-stages. B) Early adult female in the process of shedding the second-stage cuticle. C–D) Mature females.

females. This determination was based on the presence of rectal glands in second-stage immature females and the absence of rectal glands in developing males (12). Therefore, postinfective second-stage juveniles that are to become females develop rectal glands prior to the genital primordium becoming V-shaped. Thus, the presence of rectal glands indicates that those juveniles are females. The absence of rectal glands at that early stage may mean that they have not yet developed; therefore, one could not assume that juveniles without rectal glands are males.

The stylet cone, shaft, and lining of the lumen through the stylet knobs are molted. This differs from previous reports on other species of *Meloidogyne*. According to Christie and Cobb (5), Bird (3), and Siddiqui and Taylor (11), only the anterior conical portion of the stylet is shed, with the basal portion of the stylet and stylet knobs disappearing. However, my findings agree with the illustrations of *M. incognita* presented by Triantaphyllou and Hirschmann (12).

The formation of two extra esophageal lobes in females is reported for the first time.

Original descriptions of other *Meloidogyne* females illustrate the esophagi as tri-lobed glands or as an amorphous mass below the metacorpus. Bird (3) made careful examination of the esophageal region of *M. javanica* throughout juvenile development and reported only one lobe in the esophagus. Chitwood's (4) illustrations showed three lobes.

Baldwin and Sasser (2) and Eisenback et al. (6) found that the dorsal gland orifice of various species of *Meloidogyne* branched into three channels. The two extra glands described in this study have always been found in close association with the dorsal esophageal gland and most likely account for the additional two branches of the dorsal gland orifice reported by others. This indicates that those two extra esophageal glands may supplement secretions by the dorsal esophageal gland and may be the site of the products that keep the nurse cells functional. In a comparison study, I found five glands present in *M. incognita, M. arenaria,* and *M. javanica.*

Andrássy (1) stated that the five-gland condition represents a more advanced evolutionary state. The sedentary parasitic feeding habit of *Meloidogyne* spp. can be interpreted as one of the most advanced evolutionary states of parasitism. The high degree of specialization in feeding habit of these nematodes undoubtedly requires a highly specialized system to keep the nurse cells functional. A five-gland esophageal condition may aid in the digestive process by increasing qualitatively and quantitatively the digestive enzymes secreted by those glands.

Striking differences occur between second-stage juveniles and adult females of *Meloidogyne* spp. One of these differences is the position of the excretory pore. In the second-stage juveniles, the pore is usually posterior to the metacorpus, while in the adult females it is usually anterior to the metacorpus adjacent to the stylet knobs. Christie and Cobb (5) illustrated the excretory pore of adult females opposite the metacorpus. In adult *M. cruciani* the excretory pore is opposite the procorpus, which agrees with the illustrations of Chitwood (4), Bird (3), and Triantaphyllou and Hirschmann (12) for other species of *Meloidogyne.* Siddiqui and Taylor (11) did not illustrate an excretory system in *M. naasi* and did not mention the location of the excretory pore. Bird (3) examined the excretory pore and duct of *M. javanica* throughout the juvenile development but made no mention of the changes in position of the excretory pore.

Daily examinations of the developmental stages reveal the transformation that takes place in the position of the excretory pore. In the late second-stage juvenile the excretory pore is posterior to the metacorpus with the duct directed posteriorly for some distance. Immediately after the second molt the duct was observed directed anteriorly. Subsequently, in the late third- and fourth-stage juveniles, the duct was observed seemingly attached to the body opposite the procorpus. In the young adult female the old excretory pore was posterior to the metacorpus with its duct directed anteriorly, seemingly attached to the adult female body opposite the procorpus where a new excretory pore formed with a new duct directed posteriorly. After the final molt, the excretory pore and duct became very prominent, ending in a renette-type cell. Observation of the excretory gland was possible in the adult female immediately after the last molt and before enlargement. In older females the body is full of fat globules and ovaries, making it impossible to see the excretory gland. It is probable that during the molting stages the excretory system is nonfunctional, forming a new duct and a prominent excretory pore in the final stage.

The postinfection development of the gonads of *Meloidogyne cruciani* agrees in general with the descriptions and illustrations presented by Triantaphyllou and Hirschmann (12) and Siddiqui and Taylor (11). The genital primordium enlarged by cell division and migrated posteriorly. It assumed a V-shape and attached itself to the body wall, forming two branches which grew anteriorly.

Maggenti and Allen (9) gave a complete account of the formation of the rectal glands and the origin of the gelatinous matrix in *Meloidogyne* spp. They found six rectal glands present in the early postinfective second-stage juveniles before enlargement. This study supports the findings of Maggenti and Allen. Since males do not

have rectal glands, it is possible to determine the sex of the developing second-stage juveniles based on the presence of the rectal glands. Thus, sex can be determined in the very early stages of development, when the genital primordium is still in the six-cell stage and has not migrated to the posterior end of the body or assumed a V-shape.

Previous studies of the life cycle and development of the genus *Meloidogyne* revealed few details on the molting of the three cuticles by the females. Siddiqui and Taylor (11), working with *M. naasi,* were the first to mention shedding of the cuticles. They observed the old cuticles lying in the cortex in close proximity to the adult female bodies. Observations during my study indicate that the second-stage juvenile cuticle is shed while the third- and fourth-stage juvenile cuticles are either absorbed by the developing adult female or are lysed away. No feeding takes place during the third and fourth stages while the female is still enclosed within the second-, and second- and third-stage juvenile cuticles, respectively. By absorption or lysing action, the third- and fourth-stage cuticles are eliminated, while by force (mechanical action from body movements) the second-stage cuticle is broken in half and molted. Once those barriers are eliminated, the female resumes feeding.

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