Journal of Nematology 19(4):505–508. 1987.

Embryonic and Postembryonic Development of *Meloidogyne californiensis* Abdel-Rahman & Maggenti, 1987[1]

FAWZIA ABDEL-RAHMAN AND A. R. MAGGENTI[2]

Key words: *Meloidogyne californiensis*, root-knot nematode, embryogenesis, postembryogenesis, life cycle, *Scirpus robustus*, bulrush.

The embryonic and postembryonic development of root-knot nematodes (*Meloidogyne* spp.) are well known and have been studied in detail (2,4,6,7,9,10). These studies have indicated that the ontogeny of root-knot nematodes is characterized by five developmental stages separated by four molts. In 1987, Abdel-Rahman and Maggenti described a new root-knot nematode species, *Meloidogyne californiensis* on bulrush (*Scirpus robustus* Pursh) in California (1). The objective of this note is to provide information on the ontogeny of *M. californiensis*.

To study the embryonic development, *M. californiensis* egg masses were obtained from nematode-infected bulrush roots collected in a greenhouse. Twenty-three single-cell eggs were teased from the egg masses and divided into four groups. Each group was enclosed in a cavity slide filled with distilled water and placed in petri dishes lined with moist filter paper to retard evaporation. Distilled water was exchanged daily to prevent bacterial or fungal infection. Petri dishes were maintained in room temperature at 20 ± 1 C. Phases of embryonic development were recorded daily every 4–6 hours.

To study the postembryonic development, 50 7-day-old bulrush healthy seedlings were transplanted singularly in 5-cm-d clay pots containing a steam sterilized mixture of clay and sand (1:1). The soil of each pot was infested with 500 second-stage juveniles (J2) of *M. californiensis* obtained by incubating nematode-infected roots in Baermann funnels in a mist chamber. The J2 were injected in an aqueous suspension directly on the seedling roots. Twenty-four hours after root infection seedlings were removed from the pots and the root systems were washed in tap water to remove any J2 from the root surface. Single seedlings were then transplanted into 8-cm-d styrofoam pots containing the same soil mixture used above and maintained in a greenhouse at 20 ± 2 C. Two seedlings were harvested daily during the first 6 days after soil infestation and at 2-day intervals thereafter until the end of the experiment. Roots were stained with acid fuchsin in lactophenol (5), and nematodes were removed from root tissues and mounted in glycerin for microscopic examination.

Embryonic development: The first cleavage was transverse and gave rise to two equal primordial or stem cells. Six hours later, the second cleavage began with formation of a three-cell stage followed by the appearance of the four-cell stage 13 hours after the first cleavage. The third cleavage with formation of the six-cell stage, followed by the eight-cell stage, occurred about 19–20 hours after the second cleavage. The fourth cleavage, resulting in the 16-cell stage, occurred 16 hours later. The blastula and gastrula stages required 56–128 and 150 hours after first cleavage. The "tadpole" stage, starting 9–10 days after the first cleavage, marked the beginning of the development of the first-stage juvenile (J1). The J1 was fully developed 11–16 days after first cleavage. The J2 appeared 17–22 days after the first cleavage and emerged from the eggshell 20–23 days from first cleavage.

Postembryonic development: Forty-eight hours after soil infestation, vermiform J2 were found lying in the cortex parallel to

Received for publication 7 October 1985.

[1] Portion of a Ph.D. dissertation of first author.

[2] Postdoctoral Fellow and Nematologist, Department of Nematology, University of California, Davis, CA 95616.

TABLE 1. Development of *Meloidogyne californiensis* second-stage juveniles (J2).

Character	Infective J2			Female J2 11–12 days postinoculation			Male J2 11–12 days postinoculation		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Stylet	11–13	12.2	0.63	12–14	13.45	0.77	14–15	14.33	0.57
Dorsal esophageal gland orifice from spear base (DEGO)	1–3	2.8							
Distance from anterior extremity to metacorpus valve	55–70	61.36	4.82	58–60	59.93	1.67	62–68	64.66	3.05
Metacorpus (length)	12–14	12.73	0.83	20–22	20.80	1.05	20–23	21.66	1.52
Metacorpus (width)	6–9	8.1	0.78	15–17	15.73	1.10	15–17	16.33	1.15
Distance from anterior extremity to end of esophageal glands	173–264	222.9	29.10						
Body length (L)	519–597	561.6	22.43	540–564	553.3	12.22	511–598	552.33	43.66
Body width (greatest)	16–18	17.05	0.80	56–64	60.00	4.00	60–63	61.00	1.73
Tail length	82–98	88.63	4.24	96–130	113.00	24.04	94–159	119.26	34.71
Length of hyaline area to tail tip	16–30	24.23	4.37						
Length of germinal primordium, one gonad	7–11	9.98	1.66	60–79	71.73	10.28	86–101	94.00	7.54
Distance from anterior extremity to germinal primordium end	314–355	327.5	15.48	342–401	379.40	32.53	333–379	363.3	24.58
% (germinal primordium length/ body length)	1–2	1.73	0.25	10–14	12.98	2.03	16–17	17.01	0.27
% (from anterior extremity to end of germinal primordium/ body length)	51–60	54.72	3.68	63–72	68.50	4.60	63–68	65.84	2.63
a	32–33	32.95	0.89	8–10	9.2	0.4	8–10	9.2	0.96
b									
b"†	8–10	9.16	0.81	8–10	9.2	0.45	7–9	8.53	0.92
c	5–7	6.28	0.21	4–6	5.0	0.98	4–6	4.73	0.89

All measurements are in micrometers (μm).

† Calculated by dividing body length "L" by the length from anterior extremity to the end of metacorpus valve.

the vascular tissue. No changes were observed in body measurements, shape, or position of genital primordia of J2 until 5 days after penetration. Later the J2 started to feed and develop; pronounced galls were present on the roots at this time. Seven to eight days postinoculation, a significant increase in body width and metacorpus size was observed (Table 1); also the germinal primordia increased to six cells.

Second-stage juveniles were sexually differentiated 11–12 days after inoculation. The gonads of female and male J2 migrated posteriorly. Some J2 were observed to initiate molting at this stage. The molted cuticle could only be seen in the head region at this time.

The J3 (Table 2) observed 13–14 days postinfection was sausage-shaped and without the spike tail; it remained inside the cuticle of J2. The future vulva could be seen in front of the anus. No male juveniles were observed after the second molt.

J4 females were observed 15–16 days after inoculation enclosed in the J3 and J2 cuticles. The stylet was still lacking, and the valve of the metacorpus was poorly developed. Body shape continued to change (Table 2), and the future vulva was easily visible. The vulva, vagina, and uterus started to differentiate. Immediately following the J4, the young female was observed still enclosed in the cuticle of the J4, J3, and J2. The vulva, vagina, and uterus were well differentiated, as was the cuticular pattern around the vulva. The young adult female regained the stylet that it lost during the third and fourth stages.

The shedding of the larval cuticles by young females was first observed 17–18 days postinfection. The first gelatinous matrix was evident 26–27 days postinfection. No eggs were observed in the matrix at this time, but the female had reached the mature adult size. The first egg mass was observed 28–30 days postinfection. The egg mass was about ⅔, equal to, or larger than the size of the female. Each mass contained a variable number of eggs; the average was 500 eggs/mass.

The embryonic development in *M. cali-*

TABLE 2. Postinfection development of *Meloidogyne californiensis* third-stage (J3) and fourth-stage (J4) juveniles and adults.

Characters	J3 13–14 days postinoculation			J4 15–16 days postinoculation			Adult female 26–30 days postinoculation		
	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Stylet							14–17	15.2	1.42
Distance from anterior body end to end of metacorpus valve	37–48	41.93	4.85	39–59	51.74	7.39	59–79	71.56	6.75
Metacorpus (length)	17–25	21.73	2.60	22–28	26.65	2.13	28–48	34.41	3.22
Metacorpus (width)	17–19	18.63	1.42	16–28	18.35	1.64	19–31	27.07	3.09
Body width (greatest)	61–71	68.37	3.48	71–119	83.73	14.35	247–373	292.83	47.75
Length of one ♀ gonad	130–193	164.84	25.76	225–255	240.32	11.57	occupies most of the cavity		
Length of body (L)									
% (one gonad length/ body length [L])	373–444	411.22	23.94	350–427	394.2	27.23	456–584	321.31	35.66
Length of neck	33–47	41.97	5.72	54–69	61.17	4.73	108–145	122.3	11.63
a	5–7	5.91	0.47	3–5	4.53	0.71	1–3	1.82	0.34
b	9–11	9.87	0.85	6–9	7.50	1.03	6–9	7.47	0.89

All measurements are in micrometers (μm).

forniensis is similar to that described by Nagakura (7) and follows the general embryonic development in Secernentea as described by Chitwood (3). *M. californiensis* differs from *M. naasi* in that the J2 hatches easily in distilled water; the J2 of *M. naasi* became quiescent after developing and failed to hatch in distilled water (9).

The postinfection development of *Meloidogyne californiensis* resembles that described by previous investigators (2,4,9,10). However, the first adult female of *M. californiensis* was observed 17–18 days after infection, as opposed to 13–15 days with *M. incognita* (10). Siddiqui and Taylor (9) did not observe an adult female in *M. naasi* until 24 days after penetration. The postembryonic development differs from *M. graminicola* where the second molt occurred 5–6 days after inoculation (8); with *M. californiensis* this did not occur until days 13–14. The great variation in development times among individual larvae of *M. incognita* (10) was not observed in *M. californiensis.*

Literature Cited

1. Abdel-Rahman, F. H., and A. R. Maggenti. 1987. *Meloidogyne californiensis* n. sp. (Nemata: Meloidogyninae), parasitic on bulrush *Scirpus robustus* Pursh. Journal of Nematology 19:207–217.

2. Bird, A. F. 1959. Development of the root-knot nematodes *Meloidogyne javanica* (Treub) and *Meloidogyne hapla* Chitwood in the tomato. Nematologica 4:31–42.

3. Chitwood, B. G., and M. B. Chitwood. 1974. An introduction to nematology. Baltimore: University Park Press.

4. Christie, J. R., and G. S. Cobb. 1941. Notes on the life history of the root-knot nematode, *Heterodera marioni.* Proceedings of the Helminthological Society of Washington 8:23–26.

5. Goodey, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture Fisheries and Food Technical Bulletin No. 2. 47 pp.

6. Inserra, R. N., N. Vovlas, J. H. O'Bannon, and G. D. Griffin. 1985. Development of *Meloidogyne chitwoodi* on wheat. Journal of Nematology 17:322–326.

7. Nagakura, K. 1930. Ueber den Bau and die Lebensgeschichte der *Heterodera radicicola* (Greeff) Müller. Japanese Journal of Zoology 3:95–160.

8. Rao, Y. S., and P. Israel. 1973. Life history and bionomics of *Meloidogyne graminicola* the rice root-knot nematode. Indian Phytopathology XVI:333–336.12.

9. Siddiqui, I. A., and D. P. Taylor. 1970. The biology of *Meloidogyne naasi.* Nematologica 16:133–143.

10. Triantaphyllou, A. C., and H. Hirschmann. 1960. Post-infection development of *Meloidogyne incognita* Chitwood 1949 (Nematoda: Heteroderidae). Annales De L'Institut Phytopahtologique Benaki 3: 1–11.