Journal of Nematology 27(1):103–108. 1995.

Embryogenesis and Postinfection Development of *Meloidogyne konaensis*[1]

FENGRU ZHANG AND D. P. SCHMITT[2]

Abstract: The effects of temperature on embryogenesis and postinfection development in *Meloidogyne konaensis* were examined. Embryogenesis was evaluated at 5, 8, 10, 13, 16, 20, 22, 24, 26, 28, 30, 35, and 40 C. No embryonic development occurred at 5 C. Some development, although incomplete, occurred at 8 and 10 C. The rate of embryogenesis was linear from 13 to 30 C, but decreased at 35 C. The lowest egg mortality occurred at 24 C, whereas all eggs died within 24 hours at 40 C. Postinfection development was determined on coffee and tomato in a greenhouse at an average temperature of 30 C and in a growth chamber with a constant temperature of 26 C. Development of *M. konaensis* J2 to mature female required 38 and 48 days on coffee at 30 and 26 C, respectively. This process took 20 and 26 days, respectively, on tomato.

Key words: *Coffee arabica*, coffee, degree-days, embryogenesis, kona coffee root-knot nematode, life-history, life-cycle, *Lycopersicon esculentum*, *M. konaensis*, nematode, postinfection development, root-knot, tomato.

Meloidogyne species have five postembryonic developmental stages separated by four molts (4,13,16). Postinfection development of *Meloidogyne* spp. is influenced by factors such as temperature (5,14,19), soil moisture (11), pH (15), host status (10,20), pesticides, and fertilizers (17). Temperature is a major factor influencing embryogenesis and hatching, whereas temperature and host status are major regulating factors of postinfection development and reproduction (1,8,13). Computer simulation models from a temperature database have been used for predicting embryonic development and egg mortality rates (9,10).

The rate of embryogenic and postinfection development at specific temperatures varies with the species of *Meloidogyne*. The optimum temperature range for *M. hapla* Chitwood and some other cold-climate adapted species is 15–25 C, whereas 25–30 C is optimal for *M. javanica* (Treub) Chitwood and other warm-climate species (18). Very little or no development occurs in any species above 40 C or below 5 C (18).

Meloidogyne konaensis, a new species on coffee in Hawaii, was described by Eisenback, Bernard, and Schmitt in 1994 (7). This nematode was first isolated in 1991 from a coffee field at the Kona Experiment Station on the island of Hawaii. Because this is a new pest, it is necessary to know its life history and related developmental characteristics. The objectives of this research were to: i) characterize embryogenesis over a wide range of temperatures and ii) determine postinfection development of *M. konaensis* on coffee and tomato.

MATERIALS AND METHODS

Embryogenesis: A population of *Meloidogyne konaensis*, isolated from coffee at the Kona Experiment Station on the island of Hawaii, was propagated on tomato, *Lycopersicon esculentum* (L.) Mill. 'Rutgers'. Roots with egg masses were removed from the soil 2 months after infestation of the soil with this nematode and eggs were extracted using a sodium hypochlorite method (3,12). Eggs in the two-celled stage were selected as the initial stage for this study. Experiments were conducted in three series of temperatures: i) 5, 10, 16, 20, 24, 30, 35, and 40 C; ii) 20, 24, 26, 28, 30, and 35 C; iii) 5, 8, 13, and 24 C. The 24 C treatment was included in each series as a standard temperature for comparison. Treatments were replicated ten times (10

Received for publication 10 June 1994.

[1] The research reported in this publication was funded in part by the Governor's Agricultural Coordinating Committee and the Hawaiian Institute of Tropical Agriculture and Human Resources.

Journal series No. 3972 of the Hawaiian Institute of Tropical Agriculture and Human Resources.

[2] Former Graduate Research Assistant and Professor, Department of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822.

nematode eggs) for each temperature, and the experiment was performed twice.

The eggs were incubated in the dark on glass slides with five wax rings (8 mm diameter). One drop of distilled water was placed in each ring and one two-celled egg was placed into each drop of the water (five eggs/slide). The slide was placed on a BPI (Bureau of Plant Industry) watch glass half filled (five drops) with distilled water, and enclosed in a 95 × 15 mm glass petri dish containing a piece of filter paper saturated with distilled water. The drop of distilled water in each ring was exchanged with a fresh drop at 48-hour intervals.

Developmental stages of the eggs were determined by microscopic examination every 2 hours for the first 48 hours and then daily until all eggs hatched or development ceased. Stages of embryonic development were classified as: 2-, 4-, 8-cell, multicell, first-stage juvenile (J1), second-stage juvenile (J2) within the egg, hatched J2, and embryo death. Eggs were categorized as dead when contents became transparent or dark and granular.

Linear and quadratic regressions of egg development were tested for best-fit at each temperature. Development was calculated as: i) development per day for each stage and ii) development based on degree-days (DD_8) (2).

Postinfection development: M. konaensis was propagated on tomato. Eggs were extracted as described above and incubated on a 25-μm-pore sieve in an incubator at 30 C. The newly emerged J2 were collected 24 hours later and concentrated in aliquots of 500 J2 in 5 ml aqueous suspensions. Each aliquot was poured around the root system of 1-month-old coffee (*Coffea arabica* L. 'Guatemalan') and 2-week-old Rutgers tomato seedlings after temporarily removing some surface soil. Tomato and coffee roots were exposed to nematode infection for 1 and 5 days, respectively, and then washed free of soil with tap water to remove J2 that had not penetrated. (The time differential was due to the length of time required for *M. konaensis* to penetrate the two hosts). The seedlings were transplanted in 7.5-cm-d clay pots containing sterilized 0.04-mm-d sand and placed in the greenhouse (25–37 C) or in a growth chamber (26 C) with a light intensity of 380 $\mu mol/m^2s$. Three seedlings of each host were harvested daily during the first week after inoculation and at 2-day intervals thereafter until J3 developed, and checked daily until egg-laying females were observed. Roots were stained with acid fucshin (6) and dissected with the aid of a stereomicroscope to collect life stages.

Developmental stages identified were vermiform J2, swollen J2, third-stage juveniles (J3), fourth-stage juveniles (J4), young females without eggs (YF), and mature males or females with egg masses (MF). Development of *M. konaensis* in coffee and tomato roots was evaluated for 2 months.

The degree days (DD_8) required to attain each juvenile stage and to complete the entire postinfection life cycle were determined by accumulating DD_8 at different developmental stages for each temperature treatment. Degree days were used to compare the developmental rate of *M. konaensis* on coffee with that on tomato (2).

Results

Embryogenesis: The daily rate (relative amount of development in 1 day compared to total time required to complete development) of embryonic development from a two-cell stage to J2 within eggs and from J2 appearance to emergence was linear between 13 and 30 C (Fig. 1A,B). The rate of embryonic development decreased from 30 to 35 C; blastomeres died at 40 C. At 8 C, embryo development ceased at the four-cell stage. At 10 C, embryonic development progressed until the appearance of J1, which did not develop further. No development occurred at 5 C.

Cell division was temperature related (Table 1). Cleavage of the two-cell occurred at 12, 6, and 4 hours at 20, 24, and 30 C, respectively. Cleavage to the eight-cell stage was initiated at 36, 16, and 12 hours, respectively. Cleavage to the 16-cell stage occurred by 44, 26, and 18 hours

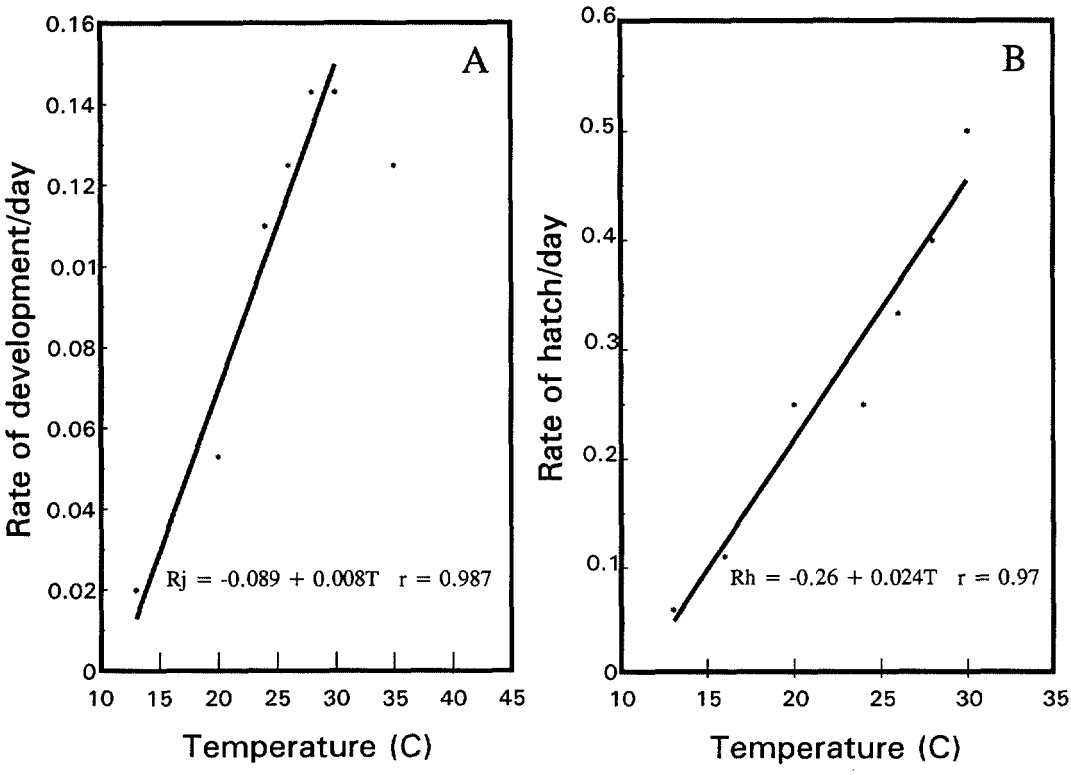

FIG. 1. Relationship between rate of *Meloidogyne konaensis* egg development (development/day) (A), and hatch (hatch/day) (B) and temperature. Rate of development/day for each temperature treatment = 1/total days required from two-cell stage to J2 within eggs at this temperature. Rate of hatch/day = 1/total days required from J2 within eggs to J2 hatched at this temperature. Rj = predicted rate of embryonic development from two-cell stage to J2 within eggs with the limits of $13 \leq T \leq 30$ C. Rh = predicted rate of hatch from J2 with the limits of $13 \leq T \leq 30$ C. * = actual data points.

later at 20, 24, and 30 C. Multicell stage eggs were observed at 38 and 30 hours at 24 and 30 C. The multicell stage within the first 48 hours did not develop at 20 C. Development at 35 C from two-cell to multicell stage paralleled that at 30 C.

The mean duration of the early embryonic stages (four-cell, eight-cell, and multicell) at temperatures from 20 to 35 C was 1 day or less (Table 2). Two days were required for the multicell stage to develop at 16 C. The more advanced developmental stages typically required 2–4 days for each stage to be completed from 24 to 35 C, and 6–21 days at 13, 16, and 20 C. The hatching process required 2–4 days at temperatures of 20 to 35 C, 9 days at 16 C, and 16 days at 13 C.

TABLE 1. Mean stage duration (hours) of *Meloidogyne konaensis* embryogenesis at 20, 24, 30, and 35 C within first 48 hours.

Stages of development	Temperature (C)			
	20	24	30	35
4-cell	12	6	4	4
8-cell	36	16	12	12
16-cell	44	26	18	20
Multicell	†	38	30	32

Values are means of 10 replications and two runs (20 observations). †: stage did not develop within 48 hours.

Embryogenesis was completed first at 30 C with hatching at 9 days (Table 2). Development to hatching was also rapid at 20 to 28 C, and 35 C. It took 21, 13, 11, 9.5, and 11 days at 20, 24, 26, 28, and 35 C, respectively. Development to hatching at 13 and 16 C required 66 and 36 days.

Degree-days requirements for completion of stages were similar within certain temperature ranges for embryogenesis. More DD_8 were required at low tempera-

TABLE 2. Mean stage duration (days) of *Meloidogyne konaensis* embryogenesis at constant temperatures.

Stages of development	Temperature (C)								
	10	13	16	20	24	26	28	30	35
4-cell	3	2	1	1	†	†	†	†	†
8-cell	6	3	1	1	1	1	1	1	1
Multicell	14	5	2	1	1	1	1	1	1
J1	102	19	11	6	3	3	2	2	3
J2	‡	21	12	8	4	3	3	3	3
Hatched J2	‡	16	9	4	4	3	2.5	2	3

Values are means of 10 replications and two runs (20 observations). †: ≤6 hours; ‡: stages did not develop.

tures (13 to 20 C) and higher temperature (35 C) than at 24 to 30 C (Table 3). The numbers of DD_8 were 195 ± 55 and 260 ± 70 for completion of development and hatching, respectively.

The optimum temperature for development and hatching was 24 C in terms of lowest percentage mortality and high hatching percentage. Mortality was also low at 13, 16, 20, and 24 C and increased from 45% at 26 C to 100% at 40 C (Table 4).

Postinfection development: Completion of postinfection development of *M. konaensis* on coffee took nearly twice as long as on tomato (Table 5). On coffee in the greenhouse, penetration and development to egg laying took 48 days. Penetration of J2 into the root tip was first detected about 120 hours after inoculation with J2. No development (change in body shape or size of genital primordia of J2) occurred until 17 days (minimum) after penetration into the roots. The first J3 developed on day 26. Galls were also evident by 26 days. Fourth-stage juveniles developed 3 days later and young females at 3 days after the J4. These females had egg masses 13 days later.

On tomato in the greenhouse, development to egg-laying female took 26 days. The J2 penetrated the roots within 24 hours after inoculation. Development of J2 and pronounced galling occurred by 5 days after penetration. J3 developed within 16 days after inoculation and J4 were observed 2 days later. Young females and egg-laying females had developed by 18 and 26 days after inoculation, respectively.

The accumulated DD_8 were similar in the growth chamber and greenhouse environments (Table 5), even though the life cycle was 6 and 10 days longer on tomato and coffee, respectively, at the cooler temperature in the growth chamber. DD_8 necessary to complete postinfection development on coffee was about double than that on tomato in both situations.

The time to develop at each stage varied among stages. The fastest progression

TABLE 3. Accumulated degree-days for mean stage duration of *Meloidogyne konaensis* egg development at constant temperatures.

Stages of development	Temperature (C)							
	13	16	20	24	26	28	30	35
4-cell	10	8	12	†	†	†	†	†
8-cell	25	16	24	16	18	20	22	27
Multicell	50	32	36	32	36	40	44	54
J1	145	120	108	80	90	80	88	135
J2	250	216	204	144	160	140	154	216
Hatched J2	330	288	252	208	198	190	198	297

Values are means of 10 replications and two runs (20 observations). †: ≤6 hours.

TABLE 4. Percentage mortality and hatch of *Meloidogyne konaensis* at a constant temperature within the range of 13–40 C.

Temperature (C)	Mortality (%)	% Hatch	
		From 2-cell	From J2 in egg
13	25	75	88
16	25	75	88
20	25	75	88
24	10	90	95
26	45	55	92
28	65	35	58
30	75	25	42
35	75	25	42
40	100	0	0

Values are means of 10 replications and two runs (20 observations).

through the life stages occurred from J4 to young female; the slowest was from J2 to J3. More than 50% of the nematode development time was spent at J2 (pre- and postinfection development). The period from young female to egg-laying female was 29% of the entire life-cycle period.

DISCUSSION

The life cycle of *M. konaensis* is similar to that of *M. javanica, M. arenaria* (Neal) Chitwood, *M. incognita,* and *M. hapla* (9,19). However, the rate of *M. konaensis* development on coffee is slow.

The minimum thermal threshold for embryonic development was 8 C. This temperature was selected because some development occurred at 8 C and none at 5 C. The maximum thermal threshold for development was between 35 and 40 C, but was not precisely determined. Based on extrapolation of the data using linear regression for temperatures of 13 to 30 C, the minimum thermal threshold is 11.13 C. This basal developmental threshold for *M. konaensis* is higher than those for *M. incognita* (8.26 C) (19) and *M. arenaria* (10.11 C) (9). Differential developmental thresholds may be important in overwinter survival and related to geographic distribution (21).

The optimum temperature for embryogenesis and hatch of *M. konaensis* is about 24 C. This conclusion is based on low mortality of eggs, the low DD_8 requirement for embryogenesis, and the high percentage of hatching. It could be argued that the optimum temperature for development is 28 or 30 C because of the faster rate of development at these higher temperatures, but it seems important to consider mortality when deciding optimum temperature.

M. konaensis had relatively high mortality during embryogenesis. The impact of this mortality on the population dynamics of *M. konaensis* needs to be explored. In addition, the inherent variation in mortality among species needs to be examined, especially to determine if warm and cold climate nematodes differ.

Tomato is clearly a much better host than coffee. Even though *M. konaensis* has been found only on coffee (Zhang and Schmitt, unpubl.), it readily develops on some plant species, such as eggplant (*Solanum melongena* L.) and cucumber (*Cucumis sativus* L.), in addition to tomato (22). More intensive surveys are needed to resolve this information on distribution.

TABLE 5. Cumulative days (CD) and accumulated degree-days (ADD) for postinfection development of *Meloidogyne konaensis* at 26 and 30 C.

Host	Temperature (C)	CD (ADD)			
		J3	J4	YF	MF
Coffee	26	26 (468)	29 (622)	31 (558)	48 (866)
	30	20 (440)	23 (506)	25 (550)	38 (836)
Tomato	26	16 (288)	18 (324)	20 (360)	26 (468)
	30	12 (264)	14 (308)	16 (352)	20 (440)

Values are means of three replications and two runs (six observations). YF: female without egg; MF: female with egg mass.

Literature Cited

1. Abdel-Rahman, F., and A. R. Maggenti. 1987. Embryonic and postembryonic development of *Meloidogyne californiensis* Abdel-Rahman & Maggenti, 1987. Journal of Nematology 19:505–508.

2. Alston, D. G., and D. P. Schmitt. 1988. Development of *Heterodera glycines* life stages as influenced by temperature. Journal of Nematology 20:366–372.

3. Barker, K. R., J. L. Townshend, G. W. Bird, I. J. Thomason, and D. W. Dickson. 1986. Determining nematode population responses to control agents. Pp. 283–296 *in* K. D. Hickey, ed. Methods for evaluating pesticides for control of plant pathogens. St. Paul, MN: American Phytopathological Society Press.

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

5. Bird, A. F. 1972. Influence of temperature on embryogenesis in *Meloidogyne javanica*. Journal of Nematology 4:206–212.

6. Daykin, M. E., and R. S. Hussey. 1985. Staining and histopathological techniques in nematodes. Pp. 39–48 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*. Vol. II: Methodology. Raleigh: North Carolina State University Graphics.

7. Eisenback, J. D., E. C. Bernard, and D. P. Schmitt. 1994. Description of the Kona coffee root-knot nematode, *Meloidogyne konaensis* n. sp. Journal of Nematology 26:363–374.

8. Ferris, H. 1976. A generalized nematode simulator based on heat-unit summation. Journal of Nematology 8:284.

9. Ferris, H., H. S. Du Vernay, and R. H. Small. 1978. Development of a soil-temperature data base on *Meloidogyne arenaria* for a simulation model. Journal of Nematology 10:39–42.

10. Ferris, H. 1978. Modification of a computer simulation model for a plant-nematode system. Journal of Nematology 10:198–201.

11. Goodell, P. B., and H. Ferris. 1989. Influence of environmental factors on the hatch and survival of *Meloidogyne incognita*. Journal of Nematology 21:328–334.

12. 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.

13. Inserra, R. N., N. Vovlas, J. H. O'Bannon, and G. D. Griffin. 1986. Development of *Meloidogyne chitwoodi* on wheat. Journal of Nematology 17:322–326.

14. Lahtinen, A. E., D. L. Trudgill, and K. Tiilkkala. 1988. Threshold temperature and minimum thermal time requirements for the complete life cycle of *Meloidogyne hapla* from northern Europe. Nematologica 34:443–451.

15. Loewenberg, J. R., T. Sullivan, and M. L. Schuster. 1960. The effect of pH and minerals on the hatching and survival of *Meloidogyne incognita incognita* larvae. Phytopathology 50:215–217.

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

17. Sipes, B. S., and D. P. Schmitt. 1989. Development of *Heterodera glycines* as affected by alachlor and fenamiphos. Journal of Nematology 21:24–32.

18. Taylor, A. L., and J. N. Sasser. 1978. Biology, identification and control of root-knot nematodes (*Meloidogyne* species). International *Meloidogyne* Project. Raleigh: North Carolina State University.

19. Vrain, T. C., and K. R. Barker. 1978. Influence of low temperature on development of *Meloidogyne incognita* and *M. hapla* eggs in egg masses. Journal of Nematology 10:311–313.

20. Wallace, H. R. 1963. The biology of plant parasitic nematodes. London: Arnold Press.

21. Wallace, H. R. 1973. Nematode ecology and plant disease. London: Arnold Press.

22. Zhang, F. R. 1994. Characterization of life-history, damage potential, and spatial pattern of *Meloidogyne konaensis*. Ph.D. dissertation. Honolulu: University of Hawaii.