Influence of Low Temperature on Development of Meloidogyne incognita and M. hapla Eggs in Egg Masses[1,2]

T. C. VRAIN and K. R. BARKER[3]

Abstract: Egg masses of *Meloidogyne incognita* and *M. hapla* were placed in soil at 10, 12, 16, and 20 C. At regular intervals, eggs from samples of egg masses were released from the gelatinous matrices and their developmental stages recorded. The number of days necessary to complete each stage from gastrulation to hatch is given for each temperature. The minimal temperature threshold for the development of eggs was computed by linear regression to be 8.26 C for *M. incognita* and 6.74 C for *M. hapla*. *Key Words:* threshold temperatures, root-knot nematode, development rate.

The survival of eggs of root-knot nematodes, *Meloidogyne* spp., in fall and winter is influenced by their stage of development and their tolerance to low temperatures. Development of eggs released from the matrix of several *Meloidogyne* species has been followed at constant (2, 8, 12) and variable temperatures (4). Because of difficulties such as the asynchronous development of eggs in the egg mass, or the opacity of the gelatinous matrix, preventing direct observation, no results have been reported on the influence of temperature on the development of *Meloidogyne* eggs in the matrix, and on the possible role of the matrix.

This study was undertaken to measure the influence of relatively low temperatures (10 to 20 C) on the survival and development in soil of eggs in egg masses of *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. hapla* Chitwood.

MATERIALS AND METHODS

Galled portions of roots with egg masses were collected and placed in cups with soil (10). Moisture tension was adjusted in all the cups at 51 cm of water (point of inflexion on the moisture characteristic of that soil). The cups were placed in boxes in a complete block design with 5 replicates per treatment, i.e., 5 replicated cups per nematode species for each harvest at each temperature. The boxes were placed in tightly closed plastic bags to avoid variation in soil moisture and maintained in temperature-controlled cabinets at one of four constant temperatures: 10, 12, 16, and 20 C. The developmental stages of the eggs in the egg masses of each species were determined at the beginning of the experiment, and after six different periods of exposure at each temperature. To span the course of development, the periods used were longer at low temperatures than at high temperatures (Table 1). The content of each cup was washed with 150 ml of water into a 250-ml centrifuge bottle and centrifuged at 400 *g* for 5 min. The supernatant was poured over a 420-μm sieve to collect the egg masses, until 20 ml remained in the bottle. The soil was resuspended in 200 ml of a 40% sucrose solution, and centrifuged at 400 *g* for 5 min. The supernatant was poured over two nested sieves (pore sizes 140 μm and 26 μm). The remaining egg masses were picked out with forceps from the top sieve. The larvae caught on the bottom sieve were washed into a beaker. The sugar solution was sieved a second time on the fine sieve (26-μm openings), and the larvae collected were added to the beaker. Eggs were released from the egg masses (5), and larvae and eggs were washed with 15 ml of water into a conical centrifuge tube. After centrifugation at 400 *g* for 3 min, the

TABLE 1. Periods of development of eggs at various temperatures.

Incubation temperature (C)	Days between inception of experiment and examination					
	t_1	t_2	t_3	t_4	t_5	t_6
10	15	30	45	60	75	89
12	12	24	34	48	61	72
16	8	16	24	32	41	48
20	5	10	15	20	24	30

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[3] Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27607. Portion of the senior author's Ph.D. dissertation. Current address: Research Station, Agriculture Canada 6660 NW Marine Drive, Vancouver, British Columbia, V6T 1X2.

supernatant was removed and the pellet resuspended in 1 ml of water. The suspension of eggs and larvae was placed in a Van Tieghem cell and examined under a microscope. The development of the eggs was recognized by changes in the number of cells, gastrulation and formation of different organs. Four hundred eggs or larvae were classified in one of 15 stages of development: embryos with 1, 2, 3–8, or 8–20 cells, blastula stage, morula stage, early and late gastrula stages, tadpole, early and late first-stage larvae, early and late second-stage larvae, hatched larvae, and abnormal (if content showed coagulation or cavitation) (10).

For each temperature the mean times of development of most stages could be calculated (6). The fourteen active stages were given values from 1 to 14, each stage j covering the range (j + 0.5) to (j – 0.5). At each harvest the median stage value of the sample of eggs was determined. The median stage of development was defined as the point on the scale of 1 to 14 where 50% of the eggs were less developed and 50% more developed, and for each sample, was found by adding the relative stage frequencies (starting at 0.5) until 50% was reached.

The median stage values of the egg samples from each harvest were plotted against time (Fig. 1), thus allowing a graphic estimation of the mean duration of developmental stages of each species at each temperature. The stage-specific rates of development, reciprocals of the mean durations of each stage, are a function of temperature. The rates of development at different temperatures were expressed as proportions of the fastest rates (at 20 C) and a least-square regression analysis gave a value of the intercept on the temperature axis, the minimal temperature threshold for development of the eggs of each species.

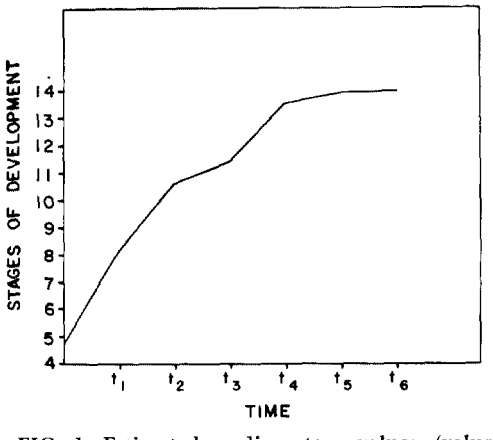

FIG. 1. Estimated median stage values (values for the curve are for *Meloidogyne hapla* eggs developing at 20 C).

RESULTS AND DISCUSSION

Preliminary studies indicated that *M. incognita* eggs did not develop at 8 C, whereas *M. hapla* eggs developed very slowly. The eggs of both species developed at 10, 12, 16, and 20 C.

Large numbers of eggs did not develop normally and died at all temperatures. Percentages of abnormal or dead eggs at 16 or 20 C were between 20 and 30% for both species at the end of the development period. At 10 and 12 C, however, the percentages of abnormal or dead eggs of *M. incognita* were very high (47 to 66%), whereas those for *M. hapla* eggs remained in the same range found at higher temperatures (9). Development to hatching of *M. hapla* eggs was slow at 10 and 12 C, and rapid at 16 and 20 C. *M. incognita* eggs did not hatch at 10 C, and development to hatching was slow at 12 C, and faster at 16 and 20 C.

The mean duration of each stage is shown in Table 2. The regression equations of the rates of development R at temperatures T between 10 and 20 C and expressed as proportions of the rates at 20 C, were: R (*M. incognita*) = −0.729 + 0.0882 (r = 0.92) with the intercept at 8.26 C, and R (*M. hapla*) = −0.529 + 0.0784T (r = 0.83) with the intercept at 6.74 C.

The percentages of abnormal *M. incognita* eggs increased at 10 and 12 C, suggesting that without a period of adaptation some of the eggs were susceptible to chilling injuries at these temperatures. It is possible, however, that some of these eggs of both species with atypical embryonic morphology, or with larvae showing abnormal coagulation of body contents, were still alive but in a quiescent state. De Guiran (3) showed that a number of eggs from *M. incognita* egg masses could suspend their development and enter a resting state even when environmental conditions were

TABLE 2. Mean stage durations of *Meloidogyne incognita* and *M. hapla* eggs developing at constant temperatures.

Developmental stages[a]	Days to complete stage[b]			
	20	16	12	10
Meloidogyne incognita				
Gb	2.5	3.3	10.0	9.5
T	2.5	3.3	9.0	15.8
L1a	2.7	5.7	16.8	37.5
L1b	4.1	6.3	11.2	23.0
L2a	4.4	6.0	—	—
L2b	6.3	5.3	—	—
Meloidogyne hapla				
M	1.4	2.0	4.0	5.5
Ga	1.5	2.0	4.0	5.5
Gb	1.7	2.0	5.5	7.5
T	2.0	3.0	7.0	5.5
L1a	2.0	3.7	13.5	16.5
L1b	5.5	3.4	7.5	10.5
L2a	2.3	3.2	8.5	20.0
L2b	2.3	3.4	—	—

[a]M, morula stage. Ga and Gb, early and late gastrula stages. T, tadpole. L1a and L1b, early and late first-stage larvae. L2a and L2b, early and late second-stage larvae.
[b]Development at 20, 16, 12, and 10 C.

favorable to development. The suspension of development was reversible, with hatching resumed after several weeks.

In previous studies the minimum temperature threshold for development of larvae was found at 10.08 C for *Meloidogyne incognita* and 8.8 C for *Meloidogyne hapla* (11), and the larval stages in eggs were more resistant to cold temperatures than either hatched larvae or embryos (10). In this context the development of eggs at lower temperatures than the larvae would have a survival value in the cold season.

The technique used in the present study also shows one possible way of overcoming the difficulty of measuring the influence of soil parameters on the rate of development of *Meloidogyne* species eggs *in situ*.

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