Development of a Soil-temperature Data Base on *Meloidogyne arenaria* for a Simulation Model

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Abstract: Models for the development rate and death rate of *Meloidogyne arenaria* relative to temperature and time were developed to improve the predictive performance of a computer simulator. About 74% of the eggs hatched fairly rapidly, whereas the remainder hatched at a lower rate and appeared to be uninfluenced by temperature shock and root exudates. Death rates of eggs were rapid at extremes of temperature (5 and 36 C) during the first week, because of sensitivity of younger eggs, and then declined and later increased gradually with time. Death rates varied little with time at optimum temperatures for survival. *Key Words:* egg development rate, egg death rate, population dynamics.

A fundamental benefit of attempts to model subsystems of the agro-ecosystem is the delineation of areas where knowledge is weak or lacking, and in determining research priorities. In developing a simulation model of a *Meloidogyne* community on grapevines (3), published data on egg development, hatch and survival (1, 10) incorporated in the model resulted in discrepancies between simulator predictions and field observations of egg and larval population levels (3). This study was done to develop a data base on egg survival and development with a population adapted to the geographic region for which predictions were being made.

MATERIALS AND METHODS

Egg development: Egg masses of a *Meloidogyne arenaria* population with 37 chromosomes (5) were dissolved by stirring in 4% commercial bleach solution (5% NaOCl) for 5 min. The eggs were rinsed in distilled water. Two-celled eggs, the earliest developmental stage readily distinguishable, were selected out with a suction device. Batches of up to 10 eggs were placed in hanging drops suspended from coverslips over Bureau of Plant Industry (BPI) watch glasses (1). Glasses were placed in each of a series of petri dishes to minimize evaporation and contamination. The petri dishes were incubated at 3 to 39 C at 3-C intervals. Developmental stages of the eggs were determined by daily examination. The number of days for development to discernible first-stage larvae and to hatching was recorded for each temperature.

This experiment constituted a data base from which quantitative models of egg development were derived. Two approaches were used: 1) the rate of development per hour from the two-cell stage to hatch was expressed as a function of temperature by regression; and 2) development was measured in terms of accumulated degree-hours (6, 7, 8, 9). A basal development threshold was determined (7, 9), and the number of degree-hours above this threshold required for hatch was calculated.

In a related study, eggs selected and placed in incubation chambers as before were subjected to fluctuating temperatures. The time taken for larval development and hatch was recorded. These data were used to test the regression and degree-hour models for their ability to predict *M. arenaria* egg development and hatch.

Because of the relatively high percentage of unhatched eggs in these studies, egg hatch and survival were studied under fluctuating temperature and in root/soil-extract solutions. The extract solutions were filtered through a 0.22-μm Millipore filter. Egg masses were placed in hatching chambers consisting of a nylon mesh disk glued between two 0.5-cm lengths of a

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1.8-cm-diam PVC pipe. Each chamber was placed in a small petri dish with either water or root/soil extract just covering the mesh. All chambers were incubated at 24 C for 4 weeks to allow most hatching to occur. Half of the petri dishes were maintained at 24 C for the duration of the experiment, and half were gradually reduced to 5 C over a 2-week period, maintained at 5 C for 4 weeks, and then increased to 24 C over another 2-week period, simulating the effect of overwintering. All treatments were incubated for a further 4 weeks at 24 C to determine the percent nonsurvival.

Egg survival: Not only does temperature regulates the rate of development of nematode eggs; it is involved also as a physiological stress factor in their survival. Five egg masses were placed in each of a series of PVC hatching chambers with distilled water in the petri dish just covering the mesh. Six petri dishes were incubated at 6 to 36 C at 3-C intervals. Each week, one petri dish was removed from each incubator. The petri-dish bottom was replaced and the hatching chamber incubated at 24 C for the next 6 weeks. The number of larvae in the original dish indicated the extent of hatch during the initial incubation. The amount of hatch in the dishes stored at 24 C was determined each week. Egg hatch dropped to a low level after 4 or 5 weeks. After 6 weeks of storage the egg masses were dissolved in 10% commercial bleach solution (2), and the unhatched eggs counted. The death percentage from the initial temperature stress was calculated on the basis of the remaining eggs and accumulated hatch.

To investigate any difference in the ability of eggs of different age groups to tolerate temperature stress, egg masses were dissolved in 4% commercial bleach for 5 min, and batches of eggs were selected that had fewer than 8 cells (<8), many cells (>8), or first-stage larvae (L_1). The eggs were placed in hanging drops (1) in BPI watch glasses at 9, 15, 27, or 33 C for 1 week. The chambers were then incubated at 24 C for 4 weeks to determine the percent survival of each stage.

RESULTS

Egg development: The hourly rate of development of eggs from 2 cells to hatch had a linear relation to temperature above 10 C (Fig. 1). Below 10 C there was little apparent development. Determined by the technique described by Milne and Du Plessis (7) and Tyler (9), the basal threshold for development was 10.11 C. The number of degree-hours above this level, determined from results at all incubation temperatures, was 4,221 ± 176.

Simulation of the number of days to hatch at fluctuating temperatures by either the rate equation or heat unit accumulations was acceptable (Table 1). At low temperatures, hatch actually occurred 1 day sooner than predicted, possibly from underestimation of the effect of fluctuating temperature on metabolic rates (6).

In the simulated overwintering study in root/soil-extract solutions, about 26% of the eggs remained unhatched after the initial 4-week period at 24 C. There were no differences between treatments in egg hatch during the initial period. The cold treatment and root/soil exudates were attempts to stimulate hatch of these eggs. At the end of the cold-shock period (12 weeks), the accumulated hatch was greater in the constant-temperature (24 C) treatments than in the cold treatments. After another 4 weeks at 24 C, however, accumu-

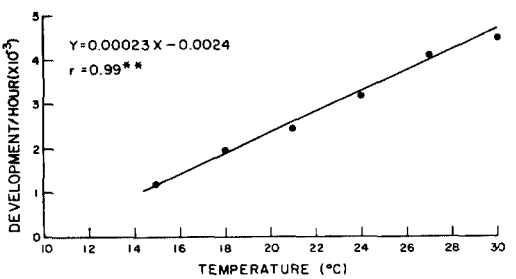

FIG. 1 .Relationship between rate of egg development (development/h) and temperature for *Meloidogyne arenaria.*

TABLE 1. Predicted hatch of eggs of *Meloidogyne arenaria* subjected to fluctuating temperature conditions by rate equations and heat units.

Treatment	Actual hatch (days)	Predicted hatch (days)
High range	11	11
Low range	21	22
Full range	12	12

lated hatch in the cold approached that in constant temperature. The percentage of eggs remaining unhatched after 16 weeks was not significantly different between treatments (Table 2).

Egg survival: An attempt to express weekly death rates (egg deaths/egg/h) as a function of temperature and exposure time over the whole period by multiple-regression techniques yielded no satisfactory correlation. When death rates were plotted against temperature by week, it was apparent that the death-rate curve outside the optimum temperature range was much steeper in the first week than in subsequent weeks. The mean death rate among temperatures was greater during the first week than in subsequent weeks (Table 3). After the second week, death rates were about constant between 18 and 24 C and increased gradually at the temperature extremes. When egg death rates for the first week were plotted against temperature (Fig. 2-A), and rates for subsequent weeks of exposure were plotted against time and temperature (Fig. 2-B), satisfactory correlations were obtained. There was high egg mortality, even in the temperature range optimum for survival, during the first week, amounting to about 36% of the eggs at 21 C. If we assume that this high initial mortality is due to experimental preparation of the eggs and that the death rate at 21 C for the first week was similar to that in subsequent weeks, the second curve in Fig. 2-A results. In this instance, the death rate is still higher at extremes of temperature during the first week than in subsequent weeks.

The survival experiment suggested that some eggs were more temperature-sensitive than others and that these eggs died during the first week of exposure. The age-group survival experiment (Table 4) suggested that eggs in the early stage of development are susceptible to 15 C and lower. Older eggs are more tolerant of low temperatures. At high temperatures there appeared little difference in the ability of the eggs to survive the stress.

TABLE 3. Duncan's multiple-range test on mean death-rate data for eggs of *Meloidogyne arenaria* at various temperatures for different weeks of exposure.

Week	Mean death rate[a]
1	0.0011 a
2	0.0007 b
3	0.0005 b
4	0.0005 b
5	0.0003 b
6	0.0003 b
LSD (P = 0.05) = 0.00035	

[a]Means with the same letter are not significantly different.

DISCUSSION

The capacity to predict egg development relative to temperature from either rate equations or accumulated heat units indicates that either approach would be satisfactory for use in simulator models. These experiments were conducted where moisture and oxygen were not limiting. There are various techniques for accumulating effective heat units (4, 6), or for use of rate equations in simulation models (3) when other conditions are suboptimal.

We hypothesize from the results that *M. arenaria* eggs of different developmental stages are differentially susceptible to temperature stress. During initial exposures to temperature stress the more sensitive eggs

TABLE 2. Percent hatch of *Meloidogyne arenaria* eggs remaining after an initial 4-week period at 24 C.[a]

Time (weeks)	24 C water	Cold-shock water	24 C root extract	Cold-shock root extract	LSD (P = 0.05)
8	8.23	4.41	16.08	6.29	1.37
12	7.68	1.19	6.04	0.16	2.09
16	1.45	15.22	0.67	14.19	4.20
Cumulative hatch	17.36	20.82	22.79	20.64	N.S.

[a]Mean of five replications. Eggs incubated at 24 C or subjected to cold treatment, in water or root extract.

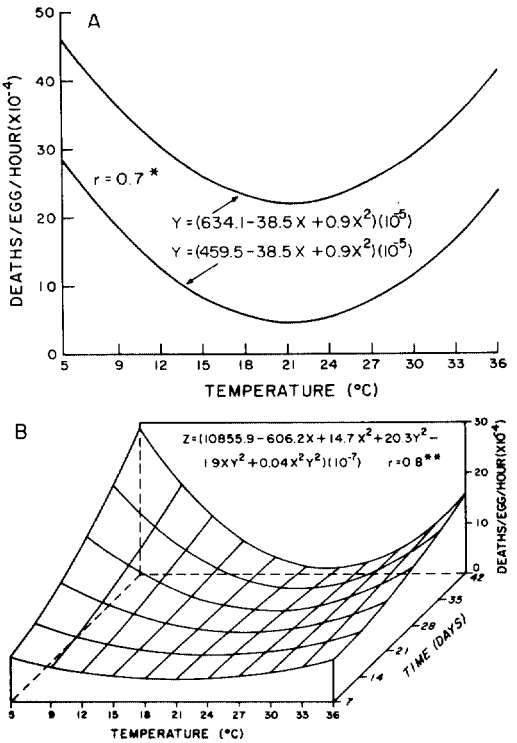

FIG. 2-(A-B). Effect of temperature and time on the death rate (egg deaths/egg/h) of eggs of *Meloidogyne arenaria*. *A)* Death rate during first week of exposure; upper curve based on actual deaths measured, and lower curve based on assumption that death rate for first week at 21 C was the same as for subsequent weeks. *B)* Death rate of eggs after first week of exposure.

are killed quickly, leaving individuals that are relatively uniformly sensitive and die at a steady rate at optimum temperatures but a gradually increasing rate at temperature extremes. Eggs between the 2-cell and gastrula stages appear to be most sensitive to the temperature stress, either from an inherent intolerance or a lack of acclimation from previous exposure to a range of temperature experiences.

TABLE 4. Percent survival of age groups of eggs of *Meloidogyne arenaria* after 1 week of exposure to various temperatures.

Exposure temperature	Developmental stage		
	<8 cells	>8 cells	L_1
9	0.0	95.5	53.1
15	0.0	83.1	80.6
27	25.9	58.1	79.5
33	26.2	39.9	29.9

LSD (P = 0.05) = 30.9

For modeling purposes, it is necessary to recognize that eggs of *M. arenaria* vary in rate of hatch. Eggs developed according to a temperature-dependent rate described by the equation

$$R = 0.000235T - 0.00235 \quad r = 0.99$$

Seventy-four percent of the eggs hatched on reaching maturity. The remaining 26% followed a different hatch pattern. They hatched at a rate of 0.000419 eggs/egg/h at 24 C after reaching maturity. Assuming the slow hatch-rate line to have the same temperature-axis intercept (10.11 C) as the egg-development line, the equation would be:

$$R = 0.00003T - 0.000305$$

This differential hatch rate may be genetically based and is probably important in survival of the nematode in field soils. We are comfortable with our assumptions on egg death rates relative to time and temperature, and plan to use the appropriate model, based on time of exposure, for simulation purposes.

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