Influence of Low Temperature on Rate of Development of *Meloidogyne incognita* and *M. hapla* Larvae[1,2]

T. C. VRAIN, K. R. BARKER, and G. I. HOLTZMAN[3]

Abstract: Development of *Meloidogyne incognita* and *M. hapla* larvae in clover roots was studied at 20, 16, 12, and 8 C in growth chambers and in the field from fall through spring, in North Carolina. Larvae of both species invaded roots and developed at 20, 16, and 12 C, but not at 8 C. The time necessary to complete the larval stages at each temperature was determined. The minimal temperature for development of *M. incognita* larvae was 10.08 C and 8.8 C for *M. hapla* larvae. In the field, soil temperature at 10 cm deep was favorable for development of larvae until the end of November, and again from February on. All stages of the nematodes survived freezing temperatures in the roots. Reproduction of both species was evident in March or April after inoculation and accumulation of 8,500 to 11,250 degree-hours. *Key Words:* ecology, threshold temperatures, survival.

The influence of temperature on the rate of development of larvae of *Meloidogyne* spp. in host roots is well documented. Studies in controlled-environment chambers (3, 17) or in temperature-controlled water tanks in greenhouses (4, 7, 14) have shown that the rates of development of all stages of *Meloidogyne* spp. are correlated positively with temperature between 15 and 30 C. Little work has been done to determine the rate of development below 15 C, or to determine the basal temperature threshold for development. Tyler (14) found that an unknown species of *Meloidogyne* developed in tomato roots at temperatures between 10 and 15 C. Griffin (7) showed that *Meloidogyne hapla* Chitwood larvae penetrate alfalfa roots and develop at 10 C. Hogger and Bird (8) found that *Meloidogyne incognita* (Kofoid and White) Chitwood reproduces in the spring in Georgia, and suggested that it may develop in the roots of winter annuals in fall and winter.

Received for publication 30 June 1977.

[1] Paper no. 5366 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C. 27607. Portion of the senior author's Ph.D. dissertation.

[2] Contribution no. J.669 of the Research Station, Research Branch, Agriculture Canada, Saint-Jean, Quebec. J3B 6Z8.

[3] Respectively graduate student, Professor of Nematology, Department of Plant Pathology, and Graduate Research Assistant, Department of Biomathematics, North Carolina State University, Raleigh, N.C. 27607. The authors express appreciation to the personnel of the N.C. State Unit of Southern Plant Environment Laboratories for their assistance.

The development of *M. incognita* and *M. hapla* larvae was compared in this study at constant temperatures between 8 and 20 C, and survival and development of the larvae in the field was studied during fall and winter in North Carolina.

MATERIALS AND METHODS

Development at constant temperatures: Ten white clover 'Dutch' *(Melilotus alba)* seedlings previously germinated on damp paper were transplanted to 10-cm-diam pots filled with a loamy sand (texture: 82% sand, 14% silt, 4% clay). The pots were placed in an environmentally controlled greenhouse with a day/night temperature regime of 22/18 C. After 5 days the seedlings were thinned to 5 per pot and inoculated with *Rhizobium trifolii.*

Depending on the subsequent temperature treatment, the plants were then allowed to adapt and grow at decreasing temperatures for various periods in the environmentally controlled greenhouse or in growth chambers (Table 1). All the plants were inoculated on the same day with freshly hatched *M. incognita* or *M. hapla* larvae. Five ml of a suspension containing 200 larvae/ml was placed in holes 3-5 cm deep in the soil. The plants were then placed at their respective treatment temperatures: 20, 16, 12, or 8 C. They were fertilized weekly with a complete nutrient

TABLE 1. Schedule of growth and adaptation periods of clover plants at decreasing temperatures and times of harvest after inoculation.

Days of growth and adaptation of plants/temperature					Treatment temperature (C)	Times of harvest after inoculation (in days)					
22/18[a]	18/14[b]	16/16[b]	12/12[b]	8/8[b]		t1	t2	t3	t4	t5	t6
10	10	—	—	10	8	37	51	68	—	—	—
10	5	—	10	—	12	18	37	51	68	79	93
10	—	10	—	—	16	12	20	28	37	44	51
15	—	—	—	—	20	6	12	18	25	31	37

[a]Day/night temperature (C) regime in environmentally controlled greenhouse.
[b]Day/night temperature (C) regime in growth chambers.

solution. In all chambers, the photoperiod was 8 h light and 16 h dark. Light intensity remained between 200 and 300 hlux during the experiment. The plants were arranged in four randomized complete blocks at each temperature.

Roots from four pots infested with either species were harvested at various intervals (Table 1), washed free of soil, boiled 5 min in 0.05% acid-fuchsin lactophenol, rinsed in water, and stored in clear lactophenol. Twenty-five nematodes were dissected out of each root system and mounted on ringed slides in lactophenol, and their developmental stages and sex were recorded as based on descriptions by Triantaphyllou and Hirschmann (13).

Regression equations of the rates of development were developed for both species. The data consisted of stage frequency counts, N_{ij}, the number of nematodes in stage j during the i^{th} harvest for each of six harvests. The relative stage frequency counts, $P_{ij} = N_{ij}/\sum_{i=1}^{6} N_{ij}$, were plotted against time (Fig. 1). Each data point, P_{ij}, is an unbiased estimator of $P_j(t_i)$, the conditional probability that an individual nematode is in stage j on day t_i, given that it has developed without premature mortality. This amounts to viewing the data as a life table.

The mean duration of any stage j is given by the area under the relative stage-frequency trend curve Pj (t) for the jth stage. This area can be estimated by the trapezoidal rule (9). It was assumed that stage j was completed if the final observation P_{6j} was equal to zero. If P_{6j} was greater than zero a hypothetical extinction time, t_7,

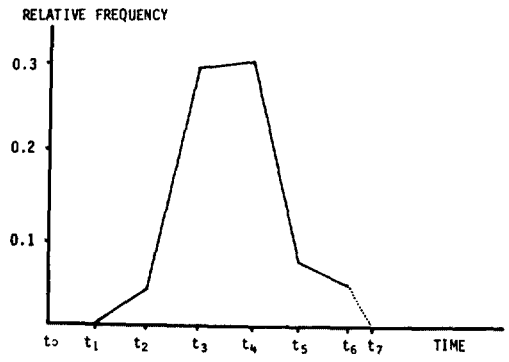

FIG. 1. Relative frequency of *Meloidogyne* larvae in roots (curve shown is for female second-stage *M. incognita* larvae developing at 20 C).

was estimated, using the values of P_{4j} to P_{6j} to extrapolate the frequency trend curve (1). In three cases, the values were not close enough to extinction to justify prediction of the unknown extinction time.

The mean time spent at each stage was calculated. The stage-specific rates of development are the reciprocals of the mean durations of the stages. For each stage, the rates of development at three temperatures were expressed as fractions of the fastest rates (at 20 C), and a least-square regression analysis gave the values of the intercept of the fitted line on the temperature axis, a linear estimate of the minimal temperature threshold for development.

The requirements of the nematodes for thermal energy can be interpreted in heat units, degree-hours above the threshold temperature for development (14), and were calculated for the two species (Table 3).

Development in the field: Microplots consisting of clay tiles (20 x 20 x 90 cm)

were established at the Central Crop Research Station, Clayton, N.C., and the Horticultural Crop Research Station, Fletcher, N.C. (107 m and 654 m elevation, respectively). The tiles were filled with topsoil representative of the test area: a loamy sand at Clayton (82% sand, 14% silt, 4% clay) and a fine sandy loam at Fletcher (40% sand, 42% silt, 18% clay). The plots were fumigated with methyl bromide (1.3 kg/10 m^2) 4 weeks before use. White clover seedlings were germinated and grown in 5-cm-diam pots in the greenhouse. After 1 week they were thinned to five seedlings per pot and inoculated with *Rhizobium trifolii*. The five seedlings were transplanted into the microplots after 4 weeks, and 1,000 *M. incognita* or *M. hapla* larvae were placed around the roots at about 10 cm deep. Two sets of clover plants were transplanted and inoculated: the first set in October, when the soil temperature at 10 cm deep was between 18 and 20 C; and the second set in November, when the temperature dropped below 12 C. The temperature at 10 cm deep was recorded continuously (soil temperature recorder, Foxboro Company, Foxboro, Mass.), and numbers of heat units were summed at each harvest. At each location, the microplots were arranged in four randomized complete blocks. The clover roots from four microplots infested with either nematode species were harvested at monthly intervals and stained, and the developmental stages of the nematodes in the root tissue were determined in the same manner as in the controlled-environment study.

RESULTS

Development at constant temperatures: Larvae of both species invaded clover roots and developed at 20, 16, and 12 C, but not at 8 C (Table 2). *Meloidogyne hapla* reproduced at the three higher temperatures, whereas *M. incognita* reproduced only at 20 C. Male nematodes of all stages were found in relatively higher numbers at 12 C than at 16 or 20 C. Almost all males observed had only one gonad. However, many cases of sex reversal were observed when, in the early phases of development of second-stage larvae, a branched genital primordium would rotate and give rise to only a single gonad. The frequencies of male nematodes at different stages were not used in the analysis, because the data were insufficient.

TABLE 2. Influence of temperature on the rate of development of larval stages of *Meloidogyne incognita* (MI) and *M. hapla* (MH).

Larval stages	Species	Number of days necessary to complete each stage[a]		
		20	16	12
Sexually undifferentiated second-stage[b]	MI	20.1	38.6	80.7
	MH	17.9	35.1	60.7
Second-stage females	MI	5.1	9.7	—
	MH	3.1	4.0	9.1
Third-and fourth-stage females	MI	3.2	—	—
	MH	2.9	5.4	9.4

[a]Time of development at constant temperature: 20, 16, or 12 C.
[b]Time of development of this stage includes migration and penetration by the larvae.

After the larvae penetrate roots the different stages of development of a given *Meloidogyne* species are likely to have similar requirements for temperature. The rates of development for all stages were used for the regression analysis, giving threshold temperatures of 10.08 C for *M. incognita* larvae development and 8.8 C for *M. hapla* larvae. Accumulated heat units required by each stage of both species are in Table 3. These results confirm, as previ-

TABLE 3. Heat units required by *Meloidogyne incognita* (MI) and *M. hapla* (MH) to complete the larval stages.

Larval stages	Species	Number of heat units		
		20 C	16 C	12 C
Sexually undifferentiated second-stage[b]	MI	4,685	5,484	3,718
	MH	4,811	6,065	4,661
Second-stage female	MI	1,189	1,378	—
	MH	834	691	699
Third- and fourth-stage females	MI	746	—	—
	MH	780	933	722

[a]Degree-hours above the threshold temperature: 10.08 C for *M. incognita*, and 8.8 C for *M. hapla*.
[b]Development of this stage includes migration and penetration by the larvae.

ously reported (13, 14, 17), that the second stage is the longest of all larval stages to complete inside the host.

Development in the field: Soil temperatures from October to April at 10 cm deep were generally higher at Clayton than at Fletcher. Average daily soil temperature remained above 9 C until December 7 at Clayton, and again from February 14 to the end of the experiment, on April 27. At Fletcher, soil temperature remained above 9 C until November 12, and again from March 10 until termination on April 8.

Larvae of both species introduced at Clayton, on October 16, invaded clover roots and developed readily. By December 5, 60% of *M. hapla* larvae and 42% of *M. incognita* larvae had matured into adult females. Many of the mature females were swollen and exuding a gelatinous matrix, indicating that they were ready to lay eggs. There was no further development of reproduction of the nematodes until the end of February. Reproduction was evident on March 11, after several females of both species had deposited eggs.

A similar though slower development took place at Fletcher when the clover seedlings were inoculated on October 13. By December 1, 40% of *M. hapla* larvae and 12% of *M. incognita* larvae had matured into adult females. Although survival of *M. hapla* was limited, reproduction took place in April. The clover plants were affected by the freezing temperatures more at Fletcher than at Clayton. Decay of roots was noticed as early as February 2, and none of the plants inoculated with *M. incognita* survived after February. The plants inoculated with *M. hapla* survived well, although many roots, galled or not, decayed during February and March and were replaced by abundant new root growth in April. Some nematodes in healthy roots were injured by cold temperatures. Their body contents were vacuolated. In some instances, the parenchyma surrounding the nematode body in the gall was hardened, and could not be softened even when treated with pectinase. In other galls, the nematode body had disintegrated, the giant cells had degenerated, and normal parenchyma filled the galls.

The pattern of development at Clayton was different when the second set of clover plants was transplanted and inoculated, on November 6. Invasion of roots by larvae of both species was low in November. *Meloidogyne incognita* larvae appeared injured in the roots, and they did not develop in March when the soil temperature rose above 10 C. Development of *M. hapla* larvae in the roots began during February. By March 11, 79% of the nematodes were either molting or were mature females. In April, most mature females were very swollen and some were depositing eggs.

When the clover plants were transplanted at Fletcher in November, penetration by the larvae of both species was very poor. All the plants were injured by the freezing temperatures, and died between January and March.

Using 8.8 C as the threshold temperature for development of *M. hapla,* the beginning of reproduction occurred after about 10,800 heat units had accumulated at Fletcher (16) and after 11,250 or 8,500 units at Clayton, depending on the date of inoculation. *Meloidogyne incognita* reproduced in the spring at Clayton only if they had been inoculated early in the fall. Using a threshold temperature of 10.08 C for development, 9,850 heat units were necessary for this species to begin to reproduce (16).

DISCUSSION

Previous studies report the developmental time of stages of *Meloidogyne* species at various temperature, and a threshold temperature can be calculated. Wong and Mai (17) give an average value of the threshold for *M. hapla* of 9.5 C in lettuce, and Davide and Triantaphyllou's data (4) give a value of 10.4 C for *M. incognita* in tomato. Tyler (14) found the minimal temperature for development of a *Meloidogyne* sp. to be between 9 and 10 C, and that 6,500 to 8,000 heat units was required for the most-rapidly developing nematodes in tomato roots to go from second-stage larvae to egg-laying females. Milne and Duplessis (10), in a field study of the development of *Meloidogyne javanica* in tobacco roots, found that about 9,000 heat units was necessary to complete development from penetration to egg laying.

The rate of development of the nematodes inside the roots not only is a function of temperature but is influenced by the

status of the plant as a host. Although it is considered a characteristic not of the plant but of the nematode, the threshold temperature calculated from the rates of development of each stage in the roots may vary with the physiological state of the plant and with changing environmental conditions.

In the present study, *M. hapla* maintained at 20 C started to reproduce after 31 days, or about 8,300 units, and about half of the females were laying eggs after 10,000 units. Periods of development were not long enough at other temperatures to measure the average time taken by the nematodes to complete their development, but at 16 C *M. hapla* females started to lay eggs after 8,800 units and at 12 C after 7,300 units had accumulated.

Results from the field agreed well with results in the temperature-controlled study. Penetration of roots and development of larvae of both species in the field were positively correlated with soil temperature—with heavy infection and rapid development resulting at Fletcher and Clayton when soil temperature was above 10 to 12 C. Infection was poor and the larvae did not develop at Clayton or Fletcher when they were placed in the soil and the temperature was below 12 C. A high proportion of nematodes were apparently able to withstand freezing temperatures for relatively long periods, but it was not determined whether a particular stage of development was more sensitive to cold than the other stages, as shown for eggs (15).

The influence of temperature below the threshold for development of these two species, and other soil factors affecting root physiology, may contribute to the variation in rates of development. After entering a stage of quiescence when the temperature is below the developmental threshold, nematodes may require a minimum number of heat units before returning to an active state.

The fact that the parenchyma cells surrounding the nematode body in some galls were not affected by pectinase suggests lignification of cell walls. Lignification in these galls would have been the result of a differential resistance of the nematodes and of the plant roots to freezing temperatures. Lignification would have occurred only after the nematodes were directly injured by the cold temperatures (6).

Galls where the nematode body had disintegrated and the giant cells degenerated were observed also in roots of other plants growing in neighboring nematode-infested plots. White clover has been shown to grow at a temperature regime of 7 C days/2 C nights (11). If plant metabolism were not affected by the quiescent nematodes at temperatures below 9 C, it is possible that the giant cells would degenerate since they would lack the necessary stimulus to function. As temperature returned to a favorable level, the nematodes became active again but their development ceased, for lack of giant cells, and they failed to survive.

It is likely that survival and development are optimum at different depths in the soil. Mean soil temperatures between October and April are relatively warm but vary greatly in the upper layers of the soil (0 to 10 cm). This situation could account for the fastest development of nematodes in roots, but would result in the most damage by cold injury. At greater depths (10 to 30 cm) development would be slower, because of lower average temperature and lower oxygen tensions, but survival would be greater than in the upper layers because the temperature would never reach 0 C or below.

Without winter weeds or a cover crop, *Meloidogyne* spp. densities decrease drastically during winter (5). The stages most likely to overwinter in the absence of a host appear to be the unhatched larvae in eggs (15). These, however, represent only a fraction of the *Meloidogyne* populations present in the soil before winter. Similar to the finding of Hogger and Bird (8) that *M. incognita* develop and reproduce in winter and spring on weeds in Georgia, this study shows that *M. incognita* and *M. hapla* can infect, develop, and reproduce in the fall and winter in North Carolina. Reproduction on any weed or crop host in early spring will increase the primary inoculum present in the soil, and the damage to the new crop (2, 12).

LITERATURE CITED

1. ASHFORD, J. R., K. L. READ, and G. G. VICKERS. 1970. A system of stochastic

models applicable to studies of animal population dynamics. J. Anim. Ecol. 39:29-50.
2. BARKER, K. R., P. B. SHOEMAKER, and L. A. NELSON. 1976. Relationships of initial population densities of Meloidogyne incognita to yield of tomato. J. Nematol. 8:232-239.
3. BIRD, A. F., and H. R. WALLACE. 1965. The influence of temperature on Meloidogyne hapla and M. javanica. Nematologica 11: 581-589.
4. DAVIDE, R. G., and A. C. TRIANTAPHYLLOU. 1967. Influence of the environment on development and sex differentiation of root-knot nematodes. 1. Effect of infection density, age of host plant, and soil temperature. Nematologica 13:102-110.
5. FERRIS, H. 1972. Population dynamics of Meloidogyne spp. in relation to the epidemiology and control of root-knot of tobacco. Ph.D. Thesis, N.C. State University, Raleigh, 95 pp.
6. GIEBEL, J. 1970. The formation of lignin like substances in roots of resistant potatoes under the influence of Heterodera rostochiensis larvae. Nematologica 16:601.
7. GRIFFIN, G. D. 1969. Effects of temperature on Meloidogyne hapla in alfalfa. Phytopathology 59:599-602.
8. HOGGER, C. H., and G. W. BIRD. 1974. Weeds and clover crops as overwintering hosts of plant parasitic nematodes of soybean and cotton in Georgia. J. Nematol. 6:142-143 (Abstr.).
9. MANLY, B. F. 1976. Extension to Kiritani and Nakasuji's method for analysing insect stage-frequency data. Res. Popul. Ecol. 17:191-199.
10. MILNE, D. L., and D. P. DUPLESSIS. 1964. Development of Meloidogyne javanica (Treub) Chitwood on tobacco under fluctuating soil temperatures. S. Afr. J. Agric. Sci. 7:673-680.
11. MITCHELL, K. J., and R. L. LUGANUS. 1960. Growth of pasture species in controlled environment: growth at low temperatures. New Zealand J. Agric. Res. 3:647-655.
12. SEINHORST, J. W. 1965. The relation between nematode density and damage to plants. Nematologica 11:137-154.
13. TRIANTAPHYLLOU, A. C., and H. H. HIRSCHMANN. 1960. Post-infection development of Meloidogyne incognita Chitwood 1949, (Nematoda, Heteroderidae). Ann. Inst. Phytopath. Benaki N.S. 3:1-11.
14. TYLER, J. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:392-415.
15. VRAIN, T. C. 1978. Influence of chilling and freezing temperatures on infectivity of Meloidogyne incognita and M. hapla. J. Nematol. 10:177-180.
16. VRAIN, T. C. 1976. Survival and development of Meloidogyne incognita and Meloidogyne hapla at low temperatures. Ph.D. Thesis, N.C. State University, Raleigh.
17. WONG, T. K., and W. F. MAI. 1973. Effect of temperature on growth, development and reproduction of Meloidogyne hapla in lettuce. J. Nematol. 5:139-142.