Effect of Temperature on Growth, Development and Reproduction of Meloidogyne hapla in Lettuce[1]

T. K. WONG[2] and W. F. MAI[3]

Abstract: Temperature was an important factor in growth, development and reproduction of *Meloidogyne hapla* in lettuce. Growth, as measured by increase in diameter of females, was not appreciably different at the intermediate (21.1 C night and 26.7 C day) and high (26.7 C night and 32.2 C day) temperature regimes, but was considerably less at the low temperature regime (15.5 C night and 21.1 C day) than at the two higher temperature regimes. Second-stage female larvae developed into adults 14 days after inoculation at the high, 18 days at the intermediate and 34 days at the low temperature regime. Eggs were observed 20 days after inoculation at the high, 26 days at the intermediate and 54 days at the low temperature regime. Number of eggs and larvae after 6 weeks was greater at the high than at the intermediate temperature regime and no eggs or larvae occurred at the low temperature regime during the observed 6 weeks. *Key Words:* Organic soil, root-knot nematode, *Lactuca sativa.*

The effect of temperature on growth, development and reproduction of several *Meloidogyne* species in various hosts is well known. Tyler (8) reported that female root-knot nematodes developed from the infective stage to the egg-laying stage in 17 days at 27.5 - 30 C and in 57 days at 15.4 C. Dropkin (3) reported that the optimum maturation temperature for *M. incognita* in soybean differed with cultivar and was between 21.5 and 25 C for 'Adams' and between 31.5 and 35 C for 'Chief'. Growth of females of *M. javanica* in tomato was studied by Bird (2) using cross-sectional area as a measure of growth. Tarjan (5) used the greatest diameter of females as a criterion for growth of five species of *Meloidogyne* females in snapdragon. A sigmoid growth curve was obtained by both workers. Thomason and Lear (6) reported that reproduction of *M. hapla* and *M. arenaria arenaria* was reduced above 30 C and 32.6 C, respectively. In alfalfa, Griffin (4) found that maximum reproduction of *M. hapla* occurred at 25 C in resistant and susceptible varieties.

Although *M. hapla* is a recognized pathogen of lettuce, little is known about the effect of temperature on the pathogen in the host. This is a study of the effect of temperature on growth, development and reproduction of *M. hapla* in lettuce growing in organic soil.

MATERIALS AND METHODS

Egg masses of *Meloidogyne hapla* Chitwood, were obtained from a greenhouse culture established from egg masses taken from 'Minetto' lettuce (*Lactuca sativa* L.) growing in organic soil (Histosol or soil of plant and animal origin) in Oswego County, New York, and maintained by periodic transfer to 'Rutgers' tomato, *Lycopersicon esculentum* Mill. Each 1-week-old lettuce seedling grown in a 90-ml paper cup at a temperature regime of 21.1 night and 26.7 C day was inoculated with 100 larvae suspended in 3 ml of water. After 2 days, roots were washed free of larvae that had failed to enter and the seedlings were transplanted into organic soil in 5-cm clay pots. Infected seedlings were grown at three temperature regimes in growth chambers with a 12-hr photoperiod at 2000 ft-c. The three temperature regimes were designated as low (15.5 C night and 21.1 C day) intermediate (21.1 night and 26.7 C day), and high (26.7 C night and 32.2 C day). The plants were fertilized weekly (100 ml of 1 part of 23% N, 19% P_2O_5, 17% K_2O : 192 parts of water). Every 2 days, two seedlings were removed and the roots washed free of soil, stained for 1-3 min with acid fuchsin in lactophenol and cleared in lactophenol. Nematodes at different stages of development were dissected and mounted in lactophenol on glass slides using layers of 'Zut' as cover glass supports to prevent crushing the swollen females. Growth of females was determined by measuring the greatest diameter of 10 randomly selected females (5). Stages of development at each

Received for publication 24 July 1972.

[1] Portion of a Ph.D. thesis submitted to Cornell University, Ithaca, New York, by the senior author.

[2] Present address: College of Agriculture, P.O. Box 203, Sungai Besi, Selangor, Malaysia.

[3] Professor of Plant Pathology, Cornell University, Ithaca, New York 14850.

We thank Dr. C. J. Eckinrode, Department of Entomology, New York State Agricultural Experiment Station, Geneva, for soil temperature data taken in organic soil near Potter, New York.

TABLE 1. Influence of temperature on development of *Meloidogyne hapla* in lettuce growing in organic soil.

Temperature (C)		Days from inoculation to:					
		2nd Stage		Female			
Night	Day	Sexually undifferentiated	Sexually differentiated	3rd	4th	5th	Egg
26.7 - 32.2 (high)		9	10	10	10	14	20
21.1 - 26.7 (intermediate)		8	10	14	14	18	26
15.5 - 21.1 (low)		14	16	24	24	34	54

sampling time were identified according to the descriptions of Triantaphyllou and Hirschmann (7).

Temperature effect on reproduction was studied in growth chambers maintained at the intermediate and high temperature regimes. For each temperature regime six 2-week-old lettuce seedlings growing in 10-cm pots were inoculated with 400 larvae suspended in 5 ml of water. To obtain uniform infection, all inoculated seedlings were grown at the intermediate temperature regime for the first three days. The plants were fertilized weekly, and after 6 weeks roots were washed, weighed and chopped. Reproduction was determined by the method described by Baldwin and Barker (1). Small pieces of infected roots were treated with 50 ml of 0.5% solution of sodium hypochlorite to dissolve the gelatinous matrix and release the eggs and larvae. The roots were stirred, allowed to stand for 5-10 min and water was added to make 200 ml. A 30-ml sample was poured through a 25-mesh sieve to remove plant tissues and two aliquots of 5 ml each were pipetted into two counting dishes. The number of eggs and larvae in each aliquot was counted and the mean number per gram of root was determined. In a second experiment the low temperature regime was included.

Sex ratios were not determined.

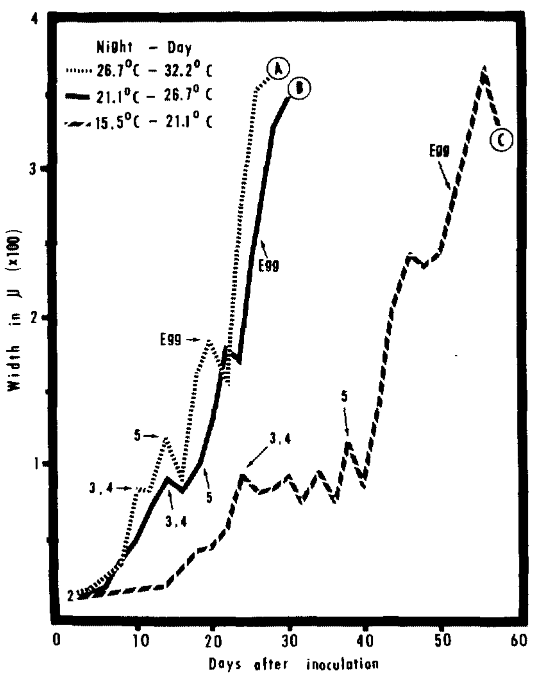

FIG. 1. The effect of three regimes of night and day temperatures on the growth (determined by measuring the greatest width) of *Meloidogyne hapla* in lettuce growing in organic soil. Numbers indicate stages of the life cycle. 3 = third stage, 4 = fourth stage, 5 = fifth stage or adult. A is high temperature regime (26.7 C night and 32.2 C day), B is intermediate temperature regime (21.1 C night and 26.7 C day) and C is low temperature regime (15.5 C night and 21.1 C day).

RESULTS

Mature females were first observed 14 days after inoculation at the high temperature regime, but did not occur until after 34 days at the low temperature regime (Table 1). Eggs were produced 6 days after fifth-stage females were first observed at the high temperature regime, but no eggs were observed in mature females at the low temperature regime until 20 days after maturity. Sexual differentiation and development to the third and fourth stages were slower at the low than at the high temperature regime. Nematode development at the intermediate regime was similar to that at the high temperature regime, but each stage in the former was delayed by 4-6 days.

At the low temperature regime, growth of *M. hapla* juveniles was slow initially but increased rapidly after 14 days (Fig. 1). Another period of slow growth occurred from the 22nd day through the 40th day, but thereafter growth was extremely rapid. The rapid growth following this period occurred after sexual differentiation. The decline in rate of growth between the 22nd and 40th day coincided with molting to form the third and fourth stage.

Growth curves of the nematode at the intermediate and at the high temperature regime were similar, but both differed considerably from that at the low temperature regime. In the higher regimes, the period of relatively slow growth was limited to about 6 days which was one-half the time of that at the low temperature regime. The rapid expansion in width of the second-stage larvae ended on the 10th day at the high but continued to the 14th day at the intermediate temperature regime. During the nonfeeding period between the appearance of the third stage and the adult, the period of relatively little growth was 2 days at the high and 6 days at the intermediate but extended to 18 days at the low temperature regime.

The number of eggs and larvae per gram of root obtained at the high temperature regime was greater than that at the intermediate temperature regime (Table 2), indicating that the former was more favorable for reproduction of *M. hapla* in lettuce. Neither eggs nor larvae were obtained during 6 weeks of observation at the low temperature regime.

DISCUSSION

An earlier report (10) indicated that invasion of lettuce roots by *M. hapla* larvae occurs in greater numbers if warm and moist soil conditions prevail at the time infested fields are seeded. Heavy infection by a high population of the nematode when lettuce seedlings were small reduced growth of the crop considerably (9). The present study indicates that following invasion of lettuce roots by second-stage larvae, growth, development and reproduction of *M. hapla* were profoundly affected by temperature. Reproduction was most rapid at the high temperature regime; at the intermediate temperature regime, reproduction was one-half to one-third that at the high temperature regime, but at the low temperature regime no reproduction occurred during 6 weeks of observation. After larval inoculation, the time required for egg production was 54 days for the low regime and 26 and 20 days, respectively, for the intermediate and high regimes. During June, July and August 1971, average minimum and maximum soil temperatures at the 10- and 15-cm depths in an organic soil were between 15.5 and 21.1 C, the lowest temperature regime included in these experiments. At these low soil temperatures it is unlikely that *M. hapla* could complete two generations in the roots of a single lettuce crop (approximately 12 weeks). During growing seasons with higher temperatures it is likely that two generations will develop.

Tyler (8) expressed the relation of time and temperature to the development of root-knot nematodes in heat units. Each centigrade degree above 10, acting for 1 hr, was counted as one effective unit. The number of units required for development from larva to egg in Tyler's data were approximately the same as those in these experiments, thus strengthening the concept proposed by Tyler.

Results from this and other experiments suggest that soil temperature may conceivably be used in conjunction with observations, such as the degree of knotting of infected roots, presence or absence of a second crop of lettuce and failure to remove infected roots after harvest, to predict the population of *M. hapla*

TABLE 2. Influence of temperature on the number of eggs and larvae produced after 6 weeks by *Meloidogyne hapla* in lettuce growing in organic soil.

Temperature (C) Night - Day	Average number of eggs and larvae/g of root after 6 weeks (six replications)[a] Experiment I (400 nematodes/plant)	Experiment II (250 nematodes/plant)
15.5 - 21.1 (low)	-[b]	0
21.1 - 26.7 (intermediate)	1662	187
26.7 - 32.2 (high)	3019	602

[a] Results significantly different at 5% level.
[b] Not conducted.

at the end of the growing season. Under New York State conditions, suitable time periods available for preplant treatments with volatile fumigant nematicides after a growing season are short and spring treatments are not recommended. Therefore, the decision whether or not to apply a soil nematicide treatment must be based on after-cropping populations of *M. hapla* in the soil. Thus, data such as those reported in this paper which increase the accuracy of estimates of these populations are of considerable economic importance.

LITERATURE CITED

1. BALDWIN, J. G. and K. R. BARKER. 1970. Host suitability of selected hybrids, varieties and inbreds of corn to populations of *Meloidogyne* species. J. Nematol. 2:345-350.
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. DROPKIN, V. H. 1963. Effect of temperature on growth of root-knot nematodes in soybeans and tobacco. Phytopathology 53:663-666.
4. GRIFFIN, G. D. 1969. Effects of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology 59:599-602.
5. TARJAN, A. C. 1952. Comparative studies of some root-knot nematodes infecting the common snapdragon, *Antirrhinum majus* L. Phytopathology 42:641-644.
6. THOMASON, I. J. and B. LEAR. 1961. Rate of reproduction of *Meloidogyne* spp. as influenced by soil temperature. Phytopathology 51:520-524.
7. TRIANTAPHYLLOU, A. C. and H. HIRSCHMANN. 1960. Post infection development of *Meloidogyne incognita* Chitwood 1949. Ann. Inst. Phytopathol. Benaki, N. S. 3:3-11.
8. TYLER, JOCELYN. 1933. Development of the root-knot nematode as affected by temperature. Hilgardia 7:391-415.
9. WONG, T. K. and W. F. MAI. 1972. Pathogenicity of *Meloidogyne hapla* to lettuce as affected by inoculum level, plant age at inoculation and temperature. J. Nematol. 5:126-129.
10. WONG, T. K. and W. F. MAI. 1973. *Meloidogyne hapla* in organic soil: Effects of environment on hatch, movement and root invasion. J. Nematol. 5:130-138.