Influence of Chilling and Freezing Temperatures on Infectivity of *Meloidogyne incognita* and *M. hapla*[1,2]

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Abstract: Egg masses and second-stage larvae of *Meloidogyne incognita* and *M. hapla* in soil were exposed to temperatures ranging from 20 to −8 C. Temperature was lowered in 2-day intervals to 16, 12, 8, 4, 0, −4, and −8 C, and the nematodes remained at 4, 0, −4, or −8 C for 18, 14, 10, or 6 days, respectively. Unhatched larvae of both species were more resistant to low temperatures than were embryonic stages. Within the eggs of *M. incognita*, 7.5% of embryos and 48% of larval stages survived 14 days at 0 C, whereas 9% of embryos and 90% of larval stages in the eggs of *M. hapla* survived 10 days at −4 C. Second-stage larvae of both species remained infective in soil at 4 or 0 C, but were injured at −4 and −8 C. Infectivity of these larvae was lower in saturated soil than in soil at 51 cm moisture tension at all temperatures. *Key Words:* Cold tolerance, survival, eggs, second-stage larvae, root-knot nematode.

Survival of eggs and larvae of the root-knot nematode, *Meloidogyne* spp., in fall and winter is influenced by their tolerance to cold temperatures. Little is known about a possible interaction between cold temperatures and low levels of oxygen in wet soils, or about the influence on survival of eggs and larvae in soil of an acclimation period (allowing rapid adjustments of homeostatic mechanisms) to cold temperature. Nusbaum (11) suggested that root-knot nematodes may adapt during autumn conditions to survive freezing temperatures. Wallace (16) showed that unhatched larvae are more resistant to low oxygen tensions than are embryos in the eggs of *Meloidogyne javanica* (Treub) Chitwood. Bergeson (1) and Sayre (12, 13) showed that *Meloidogyne hapla* Chitwood eggs and larvae were more resistant to cold temperature than those of *Meloidogyne incognita* (Kofoid and White) Chitwood.

This study was undertaken to measure the influence of low temperatures (−8 to 20 C) on the survival and infectivity of *M. incognita* and *M. hapla*, and to test the resistance of eggs and larvae, after acclimation, to chilling and freezing temperatures (−8 to 4 C) in water-saturated and unsaturated soil.

MATERIALS AND METHODS

Galled roots from tomato plants (*Lycopersicon esculentum*) 'Manapal' inoculated 6 to 8 weeks earlier with eggs of *M. incognita* or *M. hapla* were washed free of soil. Root segments bearing a single egg mass with 100 to 400 eggs each were excised so that the egg masses were undisturbed and the younger eggs were not exposed. The mature females were killed with a fine needle inserted into their body through the root tissue. Freshly hatched larvae were also obtained from egg masses on tomato roots. The eggs were released from the egg masses following a modified procedure described previously (8), and the larvae were obtained by letting the eggs hatch on 28-μm sieves (15). Ten pieces of roots each with an egg mass, or 2,000 freshly hatched larvae were placed in cups with 100 cm^3 of loamy sand (texture: 82% sand, 14% silt, 4% clay). In two-thirds of the cups the moisture tension was adjusted to 51 cm of water. In the remaining one-third of the cups, the soil was saturated. The cups were randomized in a complete block design, and placed in a temperature-controlled cabinet at 16 C.

To determine the effects of chilling or freezing temperatures on infectivity of larvae or on infectivity of larvae hatched from exposed eggs, the infectivity of larvae from 10 egg masses or 2,000 larvae was bioassayed before and after exposure to the different temperature treatments. A 2-week-old tomato Manapal seedling was transplanted into each cup and grown in a greenhouse at 24 ± 2 C. After 14 days, the roots were washed, stained in boiling 0.05% acid-fuchsin lactophenol for 5 min, rinsed in water, and destained in clear lactophenol for at least 24 h. The roots were cut in 2-to-5-cm segments, spread on 8 x 24-cm grid-lined glass plates, and examined micro-

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scopically. The number of nematodes in the roots represented the number of infective units in the inoculum.

The temperature treatments consisted of different periods of exposure at 4, 0, −4, or −8 C. The nematodes were given 6 days to adapt to the lower temperatures. After 2 days at 16 C, the temperature in the cabinet was lowered by 4 degrees every 2 days. When 4 C was reached, the cups assigned to that temperature treatment were placed in another chamber at 4 C for 18 days. Once 0 C was reached, the soil in half of the cups with a moisture tension of 51 cm of water was saturated. So, for all temperature treatments, the soil in one-third of the cups had moisture tension of 51 cm of water, in one-third it was saturated before acclimation to cold, and in one-third it was saturated after acclimation to cold.

Nematodes were exposed to either 0 C for 2 or 14 days, to −4 C for 10 days, or to −8 C for 6 days. After exposure to the treatment temperatures, the nematodes in the cups where the moisture tensions were set at the beginning of the experiment (51 or 0 cm) were allowed to adapt in a cabinet where the temperature was increased by 4 degrees every 2 days until 20 C was reached. Cups with soil saturated after acclimation to cold (on day 8) remained at each higher temperature (4, 8, 12, or 16 C) for 4 days, instead of 2, so that the time of exposure to low oxygen tension in the soil saturated before acclimation was equal to the time of exposure in the soil saturated after acclimation to cold.

After a progressive return to higher temperatures, all the cups were bioassayed on tomato. After 14 days of growth, the tomato roots were washed, stained, and examined in the same manner as the controls. All treatments were replicated 5 times. Percent infectivity was calculated from the mean number of infective units in the control, and the mean number of infective units after treatment.

To test the differential resistance of embryonic and larval stages in the eggs, the stages of development of 400 eggs in each of five cups with 10 egg masses in soil with a moisture tension of 51 cm of water were determined after an exposure of 10 days at 0 C (preceded and followed by the regular acclimation periods) for *M. incognita* and 10 days at −4 C for *M. hapla*. The eggs were released from the egg masses in 0.53% NaOCl, and examined under the microscope. Eggs were classified as embryos, larval stages, or as abnormal if content showed coagulation or cavitation, generally accompanied by browning.

RESULTS AND DISCUSSION

After exposure to cold, the composition of the egg population changed greatly (Table 1). Most of the embryos of both species were injured and discolored. About half of the *M. incognita* larvae in the eggs survived at 0 C, whereas almost all larval stages in the eggs of *M. hapla* survived at −4 C.

Unhatched larvae of both *Meloidogyne*

TABLE 1. Differential survival of embryonic and larval stages in the eggs of *Meloidogyne incognita* and *M. hapla* at chilling and freezing temperatures.

	Stages of development of eggs[a]							
	Abnormal eggs		Embryos		L1 and L2[b]		Hatched L2	
Species and treatment	Mean	se	Mean	se	Mean	se	Means	se
Meloidogyne incognita								
Before treatment	10.2	1.8	246.8	6.8	141.8	6.5	1.2	0.7
After 14 days at 0 C	305.7	9.2	18.5	3.0	66.5	6.5	9.2	0.6
LSD: $P = 0.01$	31.7		25.1		30.8		3.2	
Meloidogyne hapla								
Before treatment	5.8	1.1	262.8	10.8	143.4	11.3	1.8	0.9
After 10 days at −4 C	244.6	10.6	23.4	2.4	129.2	9.8	4.8	0.7
LSD: $P = 0.01$	39.8		37.2		N.S.		N.S.	

[a]Means of five replicates and standard error.
[b]First- and second-stage larvae in the eggs.

species survived the cold better than the embryonic and multicelled stages in the eggs. The greater resistance to low temperature of the larval stages than of the embryonic stages was observed also in eggs of *M. arenaria* (H. Ferris, personal communication) and *Ditylenchus myceliophagus* (4), indicating that the embryos in the eggs of those nematodes may not have the homeostatic mechanisms necessary to adapt to cold temperatures.

After 2 days at 0 C, 68% of *M. incognita* eggs survived and gave rise to infective larvae in unsaturated soil (Table 2), whereas no significant survival was observed at 0 C in saturated soil or below 0 C in unsaturated soil or saturated soil. The larvae of *M. incognita* remained infective at 0 C or above (Table 2) but lost their infectivity or died when exposed to freezing temperatures. At 0 C, all *M. hapla* eggs survived and gave rise to infective larvae after an exposure of 2 days, whereas only 60% did so after an exposure of 14 days (Table 2). The infectivity of larvae of both species was higher than that of larvae hatched from surviving eggs exposed to 0 C at any moisture level.

Temperature in soil between 10 and 40 cm deep rarely drops below 0 C in North Carolina. In regions where soil temperature reaches 0 C or below, *M. incognita* eggs or larvae cannot overwinter. In a field survey, Sayre (12) found that *M. incognita* could not survive in frozen soils for extended periods although *M. hapla* survived 90 days in frozen soil where a minimum temperature of −11 C was recorded. However, differences in temperature threshold for survival are to be expected between populations of different origin, as shown by Daulton and Nusbaum (6). Bergeson (1) showed that *M. incognita* larvae could not survive at 4.4 C but that *M. hapla* larvae were more resistant. Chilling-sensitive organisms usually possess membrane lipids with a high solidification temperature, becaue of a high degree of saturation (9, 10), and it is possible that an acclimation period induces chilling resistance in *M. incognita* eggs and larvae by decreasing the degree of saturation of the membrane lipids.

The eggs in egg masses are embedded in glycoproteins that constitute the gelatinous matrix (2). Unless physiological differences between unhatched and hatched larvae of *M. hapla* are responsible for the difference in survival at −4 C, the glycoproteins of the matrix may allow the eggs to supercool in the egg mass, to temperatures between −4 and −8 C.

The infectivity of larvae and larvae hatched from eggs of both *Meloidogyne* species was significantly lower in saturated soil (0 cm) than in unsaturated soil. Wallace (16) showed that *M. javanica* larvae could not survive 8 days in deoxygenated water at 27 C. The eggs of *M. javanica* were injured after 6 days in deoxygenated water at 27 C, and all the embryonic stages were

TABLE 2. Influence of chilling and freezing temperatures and soil moisture on infectivity of *Meloidogyne incognita* (MI) and *M. hapla* (MH) eggs and larvae.

Temperature and time of exposure		Species	Percent infectivity[a]					
			Eggs[b]			Larvae[c]		
			51 cm[d]	0 cm	51 → 0 cm	51 cm	0 cm	51 → 0 cm
4 C	18 days	MI				104.2a	89.7bc	37.0fg
0 C	2 days	MI	68.3de	9.5i				
0 C	14 days	MH	60.3de	2.7i	1.2i	86.0c	41.4f	7.3i
−4 C	10 days	MI	0.0i	0.0i	0.0i			
		MH	43.7f	0.4i	0.0i	2.2i	0.0i	0.0i
−8 C	6 days	MH	8.0i	0.0i	0.0i	0.0i	0.0i	0.0i

[a]Mean percentages of five replicates. Infectivity after treatment is expressed in percentages of values obtained before treatment. Mean percentages followed by a common letter do not vary significantly by Duncan's multiple-range test ($P = 0.05$).

[b]Infectivity of larvae hatched from surviving eggs in ten egg masses. Mean infectivity before treatment: 40.4% of 3,835 *M. hapla* eggs, and 62% of 1,401 *M. incognita* eggs.

[c]Mean infectivity of 2,000 larvae before treatment: 46.0% for *M. incognita*, and 42.5% for *M. hapla*.

[d]Moisture-tension levels in soil: 51 cm, tension of 51 cm of water. 0 cm, soil saturated before acclimation to cold. 51 → 0 cm, soil saturated after acclimation to cold.

killed after 1 day without oxygen (16). In this study, the eggs of both species were more susceptible to lack of oxygen than hatched larvae.

Acclimation to warm temperatures may be as necessary as that to cold. Infectivity was least when the soil was saturated after the eggs and larvae of both species had been allowed to acclimate to cold in unsaturated soil. The soil remained saturated when the temperature increased after exposure to the treatment temperatures. The lack of oxygen may have prevented normal metabolism in the nematodes (5), and the different pathways of respiration could not provide the energy necessary for the adaptation mechanisms.

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