Effect of Water, Soil Temperatures, and Exposure Times on the Survival of the Sugar Beet Cyst Nematode, *Heterodera Schachtii*

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ABSTRACT

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The effect of different combinations of temperatures and exposure times on the mortality of *Heterodera schachtii* eggs was assessed in two different experiments under laboratory conditions. In the first experiment, cysts in water were exposed to 25, 35, 37.5, 40, 42.5, 45, 47.5, 50, or 52.5°C for a maximum period of 2 h. In the second experiment, cysts in naturally infested soil were exposed to 25, 32.5, 35, 37.5, 40, 42.5, or 45°C for a minimum period of 2 h to a maximum of 2,048 h. Viability of

eggs in cysts was assessed by a hatching test in 3 mM zinc chloride solution. Viability in water was suppressed after 2-h exposure at 50°C and inhibited after 1 to 2 h at 52.5°C. Emergence of juveniles from cysts in soil was greater at the lower temperature × exposure time combinations and suppressed at higher combinations. Egg mortality started after exposure for 256 h at 40°C, 32 h at 42.5°C, and 16 h at 45°C, and 81, 31, and 7 h of exposure were necessary to kill 50% of the nematode egg population at 40, 42.5, and 45°C, respectively. The data fitted the models $P_t = P_0 10^{-\Delta t/(q + mT)}$ and $P_{T1} = P_{T0} 10^{-\Delta T/(z - pt)}$ for $m = -0.0111$, $q = 0.8238$, $z = 2.444$, and $p = -0.23$.

Additional keywords: *Beta vulgaris*, nematode mortality, soil solarization.

Characterizing the relationship between mortality of nematode eggs and temperatures requires numerous observations over a wide range of temperatures and exposure times (24). However, sensitivity to temperature stress may vary according to nematode species and life stage. Eggs of the sugar beet cyst nematode *Heterodera schachtii* Schmidt are tolerant of relatively high temperatures. Triffitt and Hurst (30) found that 30 min of exposure in water at 49°C or 10 min at 60°C inhibited juvenile emergence from eggs. Eggs within cysts did not hatch after exposure in water to 60°C for 10 s or to 47.5°C for 4 h (28). Emergence of juveniles was not completely suppressed after 28 h of exposure to 45°C. Endo (8) found that the exposure times to inhibit hatch of eggs within cysts of the soybean cyst nematode, *H. glycines* Ichinoe, were 1 s, 8 min, and 8 h at 63, 52, and 44°C, respectively. With the potato cyst nematode, *Globodera rostochiensis* Woll., Evans (9) did not observe lethal effect after exposure of cyst to 45°C for 7 h, whereas exposure to 50°C for 4 h or to 59°C for 0.5 h killed the eggs.

H. schachtii is an important pest of sugar beet, *Beta vulgaris* L. var. *saccharifera* Alefeld, especially in Europe and North America (2,6). The nematode also damages other species of the botanical families Chenopodiaceae and Cruciferae that are used both as field or vegetable crops. In Italy, *H. schachtii* is widespread (17) and recently has been found associated with severe stunting of cauliflower in Sicily (27). Because cultivars resistant to the nematode are lacking for most crop plants and because of public concern about the use of nematicides, nonchemical control methods are desirable. Among these alternatives, soil solarization is a promising option for control of several soilborne pathogens (21), including nematodes (12). Solarization is effective in Italy (4,13, 14,19). Nevertheless, its use would be more efficient with a knowledge-base on time × temperature responses (26). Therefore,

the knowledge of the relationship between the survival of the nematode and exposure times at different temperatures is fundamental. Ideally, a mathematical model that accurately describes this relationship, such as the one proposed by Greco et al. (18) for the carrot cyst nematode, *H. carotae* Jones, would be useful for practical application. That model was partly based on graphic calculations. Recently, Sasanelli and Greco (24) suggested another model based exclusively on analytical calculations and, therefore, easier to follow and to fit to the data.

The aim of this investigation was to (i) determine the mortality of *H. schachtii* in relation to exposure to heat and (ii) mathematically characterize the relationship.

MATERIALS AND METHODS

Two experiments were conducted. In the first experiment, cysts of *H. schachtii* containing eggs were placed in water and exposed to a range of temperatures and times. In the second experiment, naturally infested soil was used to test the effect of a range of temperatures and time exposures on subsequent hatching of the eggs.

The soil used to extract cysts utilized in the first experiment and for incubation in the second experiment was clay-loamy (sand 34%, silt 32.1%, clay 33.5%, and organic matter 2.4%, pH 7.6). It was collected in January from a field heavily infested by *H. schachtii* (100 eggs per g of soil) at Avezzano (province of L'Aquila), thoroughly mixed, and kept in the shade outdoors until use.

For the first experiment, cysts were extracted from 200 cm³ of soil with a Fenwick can. The average number per sample was 185 cysts (25,400 eggs). Each cohort of the approximately 185 cysts was placed into individual 2-cm-diameter sieves with 215 µm apertures and immersed in water heated to 25, 35, 37.5, 40, 42.5, 47.5, 50, and 52.5°C for 120 min. Immersion times of 15, 30, and 60 min were also tested for temperatures >40°C (Table 1). Each temperature × exposure time treatment was replicated four times. A hatching test was conducted as a means of approximating the

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viability of the eggs. At the end of the exposure times, each sieve was transferred to a 3-cm-diameter plastic petri dish (2) containing 3 ml of a 3 mM zinc chloride solution (5,20). These plastic dishes were randomly arranged in 15-cm-diameter glass petri dishes and incubated at $25 \pm 2^\circ\text{C}$ for 10 weeks. At 7-day intervals, emerged juveniles were counted and the Zn chloride solution was discarded, and fresh Zn chloride solution was added to each plastic dish.

At the end of the 10 weeks, the cysts held in each sieve were crushed using Bijloo's modified method (25) to release unhatched eggs. These eggs were counted, and the percent hatched was calculated as the number of hatched juveniles/sum of hatched juveniles + unhatched eggs. Percent unhatched was assumed to represent egg mortality.

In the second experiment, 250-cm³ plastic cups were filled with infested soil and water was added to bring the soil moisture content to 33 kPa (43% of the dry soil weight). Groups of four containers were sealed in plastic bags and placed in growth chambers. The combinations of temperatures $\times$ exposure times that were tested are provided in Table 2. The tested temperatures were selected based on the results of the first experiment. Each exposure $\times$ temperature combination was replicated four times. At the end of each exposure time, the cysts were extracted from the wet soil with the Fenwick can and incubated at $25 \pm 2^\circ\text{C}$ in a 3 mM zinc chloride solution as in the former experiment. The hatching test lasted 10 weeks or until egg hatch became negligible.

Assumed egg mortality data were subjected to probit analysis (10). Also, standard errors were calculated for each treatment. The global standard error from all time $\times$ temperature combinations was calculated using the formula

$$SD_{\text{total}} = \text{SQRT}[(n_x - 1)SD_x^2 + (n_y - 1)SD_y^2 + (n_z - 1)SD_z^2 + \dots]/(N_{\text{total}} - 1)$$

TABLE 1. Effect of 15, 30, 60, and 120-min exposure in water at different temperatures on cumulative egg hatch of *Heterodera schachtii* (% hatch)

Temperature ($^\circ\text{C}$)	Exposure time (min)			
	15 ^a	30	60	120
25	...	...	...	70.0 $\pm$ 3.31
35	...	...	...	80.0 $\pm$ 2.44
37.5	...	...	...	61.0 $\pm$ 1.23
40.0	...	...	70.7 $\pm$ 1.14	73.0 $\pm$ 2.54
42.5	...	...	74.0 $\pm$ 1.72	69.2 $\pm$ 1.51
45.0	...	65.5 $\pm$ 1.45	65.5 $\pm$ 2.08	73.7 $\pm$ 1.36
47.5	75.0 $\pm$ 1.91	79.5 $\pm$ 3.04	72.0 $\pm$ 2.62	70.0 $\pm$ 0.76
50.0	67.0 $\pm$ 2.29	69.7 $\pm$ 1.49	71.2 $\pm$ 3.08	57.7 $\pm$ 2.43
52.5	57.7 $\pm$ 3.16	51.5 $\pm$ 2.63	0.3 $\pm$ 0.02	2.5 $\pm$ 0.29
LSD 0.05	8.12	7.03	8.1	10.9
LSD 0.01	11.67	9.85	11.1	14.7

^a Values are the means of four replicates $\pm$ standard errors.

where x , y , and z represent the individual time $\times$ temperature treatments, n is the number of replicates in each treatment, and N is the total number of replicates.

To predict the efficacy of a method to control nematodes, information is necessary on the relationship between the rates of the control method and the nematode mortalities that can be achieved. Therefore, to predict the performance of soil solarization in controlling nematodes, a model describing the relationship between nematode survival and exposure times to different temperatures is necessary. Brown and Melling (3) stated that during heat sterilization the log of microorganisms survival $\times$ exposure time at a constant temperature is a linear relationship. Based on this statement, to calculate the nematode population surviving after a given exposure time to a constant temperature, Sasanelli and Greco (24) proposed the model

$$P_t = P_0 10^{-\Delta t/(q + mT)} \quad (1)$$

In this equation, P_t is the nematode population after Δt exposure time in hours; P_0 is the nematode population before the heat treatment; Δt is the exposure time above t_t (defined as the threshold exposure time $\times$ temperature below which the nematode is not killed); m and q are the angular coefficient and the known term (y -axis intercept), respectively, of the straight line that represents the function $\alpha = f(T)$; and T is the temperature in degrees Celsius to which the nematode population is exposed. The value of α corresponds to the nematode decimal reduction time, i.e., the exposure time, above the threshold time at a given constant temperature, required to reduce the initial nematode population (P_0) to 1/10. It is assumed that α is constant for each nematode species and temperature; therefore $\alpha = f(T)$.

To calculate values of α , for each of the temperatures that reduced egg hatch, log of percent nematode survival observed at exposure times $>$ threshold exposure time (defined as the exposure times to a given temperature up to which no hatch reduction occurred) were plotted against exposure times at the given temperature, and a straight line was fitted according to Sasanelli and Greco (24).

Values of α were then plotted against temperature to derive the equation representing $\alpha = f(T)$. In this equation, the slope and the y intercept correspond to m and q in equation 1.

A similar model was proposed to estimate the nematode population after a constant exposure time to varying temperatures (24). In this case, the model is

$$P_{T1} = P_{T0} 10^{-\Delta T/(z - pt)} \quad (2)$$

where P_{T1} and P_{T0} are the nematode populations (at the same exposure time) at T_1 and T_0 ; ΔT is the temperature increase ($T_1 - T_0$); p and z are the angular coefficient and the known term (y intercept), respectively, of the straight line that represents the function $\beta = f(t)$ or $\beta = f(\log t)$; and t is time of nematode population exposure to temperature T_1 . The value of β corresponds to nematode decimal reduction temperature, the temperature in-

TABLE 2. Percent cumulative hatch of *Heterodera schachtii* after soil exposure at different time $\times$ temperature combinations

Exposure (hours)	Temperatures ($^\circ\text{C}$)						
	25 ^a	32.5	35	37.5	40	42.5	45
2	53.7 $\pm$ 2.35	...	...	...	...	...	...
4	58.3 $\pm$ 2.21	...	...	...	...	...	...
8	70.4 $\pm$ 2.77	...	...	...	...	...	48.0 $\pm$ 3.18
16	75.8 $\pm$ 3.18	...	...	...	...	...	8.9 $\pm$ 0.66
32	76.3 $\pm$ 2.71	...	...	...	82.1 $\pm$ 3.15	47.1 $\pm$ 3.21	0.3 $\pm$ 0.06
64	60.8 $\pm$ 2.59	...	...	...	48.8 $\pm$ 1.52	0.6 $\pm$ 0.09	0.2 $\pm$ 0.05
128	64.5 $\pm$ 2.31	81.0 $\pm$ 1.37	76.9 $\pm$ 2.40	76.3 $\pm$ 2.78	39.7 $\pm$ 1.34	0.1 $\pm$ 0.04	0.1 $\pm$ 0.06
256	60.9 $\pm$ 1.67	75.5 $\pm$ 1.49	72.9 $\pm$ 1.94	77.9 $\pm$ 1.82	1.1 $\pm$ 0.08	0.1 $\pm$ 0.01	0.0 $\pm$ 0.00
512	81.7 $\pm$ 1.64	81.3 $\pm$ 2.28	84.0 $\pm$ 1.61	77.2 $\pm$ 3.44	1.4 $\pm$ 0.15	0.1 $\pm$ 0.01	0.0 $\pm$ 0.00
1,024	76.1 $\pm$ 1.62	69.5 $\pm$ 0.75	75.6 $\pm$ 1.51	75.4 $\pm$ 3.11	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
2,048	83.5 $\pm$ 1.79	88.1 $\pm$ 1.08	86.9 $\pm$ 2.36	61.5 $\pm$ 1.61	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00

^a Values are the means of four replicates $\pm$ standard errors.

crease (at the same exposure time) required to reduce the nematode population occurring at T_0 to 1/10. Also, β can be calculated as the inverse of the angular coefficient of the straight line characterizing the function $\log_{10} P = f(T)$. Its value is assumed to be the same for each nematode species and exposure time. Values of β were determined by plotting log nematode survivals against given temperatures at each exposure time, as they correspond to the inverse of the slopes of the straight lines ($\beta = -1/b$). Values of β were then plotted against log exposure times to derive the equation of the straight line representing $\beta = f(t)$. In this equation, the slope and the y intercept are p and z in equation 2.

Therefore, to characterize the relationship between survival of *H. schachtii* eggs as affected by a range of temperatures and exposure times, the logs of egg survival observed in the second experiment were used to calculate q and m and p and z in equation 1 and equation 2, respectively.

RESULTS

The standard error was rather low for all data, indicating little variability among replicates. Values of global standard error were 1.85 and 1.57 for the experiment in water and in soil, respectively.

Exposure of cysts for 1 to 2 h in water at 52.5°C completely inhibited egg hatch but no hatch reduction was observed after exposure for 15 or 30 min. After 2 h at 50°C, emergence of juveniles was delayed during the first 4 weeks but reached 58% by the end of the hatching test. Cysts were not affected by 2 h in water at 25 to 47.5°C and had similar final hatch percents and hatching patterns (Table 1). Most of the juveniles emerged during the first 5 weeks and only a few thereafter. Total cumulative hatch was 70 to 80%.

In the second experiment, cysts at 25°C, considered the reference temperature, had rather low final hatch percent (53.7 to 58.3%) when exposed for 2 to 4 h and much higher juvenile emergence (83.5%) at the longest exposure times (2,048 h) (Table 2). The suppressive effect of the heat started clearly at 40°C. At this temperature, egg hatch was not reduced after a 32-h exposure, but after 64 to 128 h, emergence of juveniles was delayed by 1 week and reached only 40 to 49% by the end of the incubation period. Longer exposure at this temperature resulted in complete inhibition of egg hatch. At 42.5°C, hatch was again delayed by 1 week after 32-h exposure and was 47% at the end of the test; there was no hatch when exposure lasted ≥ 64 h. At 45°C, total emergence was 48% after 8-h exposure, negligible (9%) after 16 h, and completely inhibited at longer exposure times. No reduction of egg hatch occurred at 37.5°C up to 1,024-h exposure and only a little at 2,048-h exposure.

At 32.5 and 35°C, maximum egg hatch was obtained at 2,048 h (87 to 88%), but total hatch percent was always above 70% at all shorter exposure times. Most of the juveniles emerged during the first 4 weeks (82 to 85%) and much fewer during the remaining 6 weeks.

Probit analysis was conducted only on data for temperatures $\geq 40^\circ\text{C}$ because no egg mortality occurred at lower temperatures. The correlation coefficients (r) of the linear regression equation relating percentage of emerged juveniles to exposure times were highly significant for all three temperatures (40, 42.5, and 45°C) (Fig. 1). Exposure times required to attain a 50% mortality were 81 h at 40°C, 31 h at 42.5°C, and 7 h at 45°C, and a 90% mortality could be reached after 170-, 44-, and 14-h exposure at 40, 42.5, and 45°C, respectively.

Plotting log egg hatch of each suppressive temperature against log of exposure times causing reduction of egg hatch (64 to 1,024 h at 40°C; 32 to 512 h at 42.5°C; and 8 to 128 h at 45°C) gave equations of the straight lines representing this relationship at these temperatures. These equations (Fig. 2) enabled the calculation of α values that were 0.3993 at 40°C, 0.318 at 42.5°C, and 0.344 at 45°C. It must be noted that these values are all derived

from regression straight lines with a highly significant regression coefficient. The α values were plotted against temperature (Fig. 3), and values of m and q of -0.011 and 0.8238, respectively, were derived from the straight line representing this relationship. Therefore, equation 1 for *H. schachtii* is

$$P_t = P_0 10^{-\Delta t / (0.8238 - 0.011T)}$$

Similarly, values calculated for β were 2.079 at 32 h, 2.069 at 64 h, 1.920 at 128 h, 1.809 at 256 h, and 1.853 at 512 h (Fig. 4). These values were then plotted against log exposure time (Fig. 5) to derive the equation representing $\beta = f(\log t)$, in which the slope (-0.236) and the y intercept (2.444) correspond to p and z in equation 2, which becomes

$$P_{T1} = P_{T0} 10^{-\Delta T / (2.444 + 0.236r)}$$

Exposure times predicted by the model for different levels of mortality are lower than values deriving from probit analysis at 40°C, but the result is always higher at 42.5 and 45°C (Table 3). However, differences were not statistically significant according to Student's t test. The correlation between estimated exposure times and mortality levels yielded an R^2 of approximately 0.90 at all temperatures in the proposed model versus 0.95 to 0.98 in probit analysis.

Fig. 1. Relation between exposure time and probit mortality of eggs of *Heterodera schachtii* in soil at 40, 42.5, and 45°C (C.L. = confidence limit).

DISCUSSION

Eggs of cyst nematodes may undergo a dormancy or diapause period during which egg hatch is nil or poor. This is well known for the cereal cyst nematode, *H. avenae* Woll. (11) and potato cyst

nematode *G. rostochiensis* (23), under temperate climatic conditions. Therefore, one may question whether the lack of or poor hatch of eggs exposed to rather high temperature is due to lethal effect or to induction of dormancy. Greco et al. (15) did not observe a clear dormancy of *H. schachtii* eggs from the same area although Zheng and Ferris (32) reported significant differences in hatch of eggs of this nematodes produced or stored under different environmental conditions. From Table 2 it is evident that the exposure to temperature caused an increase in percent egg

Fig. 2. Regression straight lines describing the relationship between log nematode population and log exposure time at 40, 42.5, and 45°C for calculation of α values (nematode decimal reduction time).

Fig. 3. Relationship between temperature and values of α and calculation of the parameters q and m in equation 1.

Fig. 4. Regression straight lines describing the relationship between log nematode population and temperatures at 32, 64, 128, 256, and 512 h for calculation of β values (nematode decimal reduction temperature).

hatch at low temperature $\times$ exposure time combinations and a sharp reduction at higher combinations. This would exclude an induction of dormancy due to temperature effect in our experiments. Nevertheless, the infested soil was collected from the field at the end of January, when cysts were at least 3 to 4 months old, and the hatching tests were conducted for a period long enough to interrupt dormancy (a minimum of 10 weeks in the second experiment). Therefore, it is reasonable to assume that nonhatched eggs were dead due to the lethal effect of exposure to temperature. The fact that 100% egg hatch was not attained even at low temperature $\times$ exposure combination can be attributed to the presence of nonviable eggs and/or eggs infected by fungi, 12% in Greco et al. (15). Moreover, to avoid loss of hatch stimulation with aging, we used a solution of an inorganic compound (3 mM zinc chloride) as hatching agent, instead of host plant root diffusate. This compound is more stable and known to induce hatching similar to that of sugar beet root diffusate.

According to the results of the first experiment, a 2-h exposure of cysts in water up to 47.5°C did not affect hatchability. At 50°C juvenile emergence was only delayed and partially suppressed. Temperatures above 50°C were lethal (caused at least 80% egg mortality) only if exposure was for at least 2 h.

The experiment with infested soil demonstrated the increasing effects of different short heat treatments on hatchability of *H. schachtii*, as previously observed for *H. carotae* (18). An increasing effect was found at low temperature and exposure time combinations and a decreasing effect at higher combinations. The decreasing effect is the only one of practical interest. For *H. schachtii*, at least 64-h exposure at 40°C is required to achieve a satisfactory level of control. These findings are similar to those of Steele (28). This temperature can be achieved in solarized soil at depths of up to 10 cm in many areas. However, in southern Italy, temperatures ranging from 38 to 40°C have also been recorded at 30 cm deep in solarized soil. The potential for control of *H. schachtii* by soil solarization is good in these areas.

Fig. 5. Relationship between log exposure time and values of β and calculation of the parameters z and p in equation 2.

TABLE 3. Comparison of exposure times estimated by probit analysis and the proposed model to achieve different levels of mortality of eggs of *Heterodera schachtii* at 40, 42.5, and 45°C

Mortality (%)	Exposure time (h)					
	40°C		42.5°C		45°C	
	Probit	Model	Probit	Model	Probit	Model
50	81.0	84.4	31.0	40.9	7.2	10.0
60	92.5	91.0	33.6	44.3	9.0	10.8
70	108.1	101.6	36.3	49.1	10.1	11.9
80	129.7	118.7	39.9	56.8	11.4	13.6
90	167.0	154.9	45.3	72.7	13.7	17.0
R ²	0.946	0.898	0.973	0.905	0.984	0.915

The model is satisfactory for temperatures ≥ 40 , but less so at lower temperatures and can be used to derive the duration of the exposure to soil temperatures necessary to reduce a given population of *H. schachtii* to a desired level. In our case, reducing the initial population density of 100 eggs per g of soil to 1.8 eggs per g of soil, corresponding to the tolerance limit of sugar beet for *H. schachtii* in Italy (16), would require an exposure to 45°C for 29 h, 42.5°C for 65 h, or 40°C for 147 h. The exposure would be reduced to 7 h at 47.5°C and to about 3 h at 50°C. Such temperatures can be reached in the top 10 cm of solarized soil (1,7).

Several models are available to predict soil temperature in the soil profile under different mulches, including plastic films, which are based on available meteorological data and soil and mulch properties (22,29,31). These models are useful to predict soil temperature under different climatic and soil conditions and thus estimate the effectiveness of soil solarization using our model. Prior to solarization, the soil is ploughed to a depth of about 30 cm. Therefore, because cysts are not motile, the population of cyst nematodes is more uniform throughout the top 30 cm of the soil profile.

Comparing these results with those of previous studies suggests that *H. schachtii* is much more sensitive than *H. carotae* to heat stress (18). For instance, to reduce nematode population to 1.5% of the initial density, an exposure of 24 h at 45°C is required for *H. schachtii*, but 60 h is required for *H. carotae*. At the same temperature, a reduction of nematode population to 3% would require 19-h exposure for *H. schachtii* and 52 h for *H. carotae*. Differences in metabolism and ecological amplitude may account for the different sensitivity of the two nematode species. The difference observed in the survival ability of these two cyst nematode species, to the same range of temperatures $\times$ exposure times, suggests that parameters cannot be used interchangeably among nematode species. Parameter values must be determined experimentally for each species.

Finally, the models described here could also fit survival data of other soilborne microorganisms in relation to exposures to a range of temperatures.

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