The Effect of Temperature and Moisture on the Survival of Heterodera glycines in the Absence of a Host

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Abstract: Soybean-cyst nematode larvae survived in water up to 630 days, depending on incubation temperature. Most larvae were killed when ice crystals formed in water, and all died after 1 day at 40 C. At temperatures of 0, 4, 8 and 12 C, larvae survived for the duration of the experiments (630 days). From 16 to 36 C, survival was inversely correlated with temperature. In naturally infested soil, nematode survival was similar but more extended and related to moisture level. Larvae survived 7-19 months in flooded soil, 29-38 months in dry soil, and for 90 months in soil maintained near its field capacity.

Heterodera glycines is an obligate parasite with a broad host range (6, 9, 10, 11, 12, 14, 15). Like most cyst-forming nematodes, it is able to survive for extended periods in the absence of a host. Temperature and moisture affect survival of free larvae or contents of cyst bodies. Eggs in *H. rostochiensis* cysts survived several months in dry soil at room humidity (8). Larval emergence from *H. major* cysts was considerable after 3 months' storage in air-dry soil, but ceased after 6 months (1). When *H. major* cysts were removed from the soil and dried, larval emergence ceased in a matter of hours (5, 19). Lewis and Mai (7) showed that moisture level changes the effect of temperature on survival of *H. rostochiensis* eggs and larvae within cysts, and that alternating temperature had a more adverse effect than constant temperature. *H. schachtii* larvae survived in water for 6 months, and, in soil without plants in the greenhouse, larvae survived for 12 months (3). *H. schachtii* survived 3 months in fallow surface soil in which the temperature went as high as 52 C (17). Drying reduced the viability of the contents of *H. schachtii* cysts, but the reduction appeared to be a function of rate of drying rather than dryness (18).

The results of studies on the effect of temperature, moisture, soil type, cyst condition, aeration and other factors on various stages of the life cycle and survival of *Heterodera glycines* have been reported (2, 4, 6, 13, 16, 19). This is a report of studies on the effect of a range of temperatures and various moisture levels on the survival of *H. glycines* eggs and larvae in the absence of a host plant.

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MATERIALS AND METHODS

In order to study the effect of temperature on the survival of larvae, aliquots of freshly hatched larvae were placed in syracuse watch glasses held at various temperatures from -4 to 40 C. Water was added periodically to maintain a water level of 1-2 mm. The samples were checked at intervals, and various tests used to determine whether or not the larvae were alive and active. The first test was to touch them with a probe, and if this produced no movement, a few larvae were cut to determine whether the body contents flowed. Soybean seedlings were inoculated at intervals and later processed for the recovery of mature females to determine whether the larvae were capable of penetration and development.

We checked survival of nematodes in soil as influenced by temperature and soil moisture by placing large samples of infested soil in plastic bags at the various temperatures. One series of samples was kept moist throughout the test period, another set was allowed to dry to air-dryness. A sample was taken at intervals, processed by roiling and sieving and the residue on the sieve placed on a funnel to check for larval recovery. When the number of larvae recovered from the funnels dropped to a low level, samples were planted to soybean, *Glycine max* L. 'Lee', to determine whether surviving larvae would penetrate roots and develop.

The influence of four moisture levels on survival of *H. glycines* in the soil was tested in the greenhouse using natuarally infested soil of two types, sandy and clay, potted in 7.6-cm pots and maintained at the following moisture levels: (i) air dry (no water added); (ii) watered daily to keep at field capacity; (iii) saturated by setting pots in a basin of water; and (iv) submerged in water at all times. Samples were taken at intervals for a bioassay of nematode survival. A Baermann funnel assay at the

beginning of the test indicated a population of 500 viable eggs and largae/100 cc of soil.

RESULTS

Heterodera glycines larvae in water became inactive after a short period of incubation, but could be induced to move by a touch with a probe. Larvae in water remained infective for only 1 day at -4 and 40 C (Table 1), although they survived for somewhat longer. From 0 to 12 C, larvae survived for approximately 630 days, at which time samples were depleted. Survival at 16-36 C was inversely correlated with the temperature. When cysts were removed from the soil and incubated in water at 40 C, larvae were recovered after 2 days but not after 1 week (13).

Temperature had a similar effect on the survival of a natural population in the soil, but nematodes survived for a much longer period (Table 2). Freezing did not immediately destroy the nematodes, and at 40 C in dry soil a positive bioassay was obtained at 50 days. At 4, 8, 12, 16 and 20 C, nematodes survived the entire test period (6-8 years). Above 20 C, the survival time decreased as the temperature increased. At 20 C, and below survival was better in the moist soil. From 24 to 36 C, survival was better in the dry soil.

TABLE 1. Survival of *Heterodera glycines* larvae in water at various temperatures.

Temp (C)	Days to: Last movement[a]	Last infection	Last turgid
-4	26[b]	1	60
0	630[c]	630	630
4	630	630	630
8	630	630	630
12	630	630	630
16	190	530	570
20	190	310	420
24	66	120	280
28	44	60	210
32	30	44	150
36	12	15	35
40	1/4	1 1/6	7

[a]Larvae became inactive after a short period, but could be induced to move by a touch with a pulp canal file. When movement was no longer obtained, a biological check and a turgidity test was used to determine survival.

[b]Most larvae died immediately upon the formation of ice crystals.

[c]Samples at 0, 4, 8 and 12 C were depleted at 630 days.

TABLE 2. The effect of temperature and soil moisture on survival of *Heterodera glycines* in soil.

Temp (C)	Months survival in: Moist soil[a] Baermann funnel	Moist soil[a] Bioassay	Dry soil Baermann funnel	Dry soil Bioassay
-4	49[b,c]	–	–	–
4	96	96	84	84
8	84	84	72	72
12	84	84	72	72
16	84	84	72	72
20	84	84	72	72
24	26	23	30	30
28	22	17	30	30
32	22	17	30	11
36	6	6	16	11
40	4/15	4/15	4/15	1 2/3

[a]Soil was kept in plastic bags, and moisture added periodically; the dry soil was allowed to dry and water was never added.

[b]At -4 C, few larvae were found throughout the test.

[c]At 4, 8, 12, 16 and 20 C, larvae were recovered and the bioassay was positive when the supply of infested soil was depleted.

Survival of *H. glycines* definitely was related to moisture level (Table 3). Infection was obtained after 7-19 months under flooded conditions, and 29-38 months under air dry conditions. In saturated soil, infection occurred for as long as 63 months and for 90 months in soil watered daily.

DISCUSSION

At 36-40 C and below 20 C, a delayed reading at room temperature following treatment was often required. The turgidity test, in which the nematode was punctured to determine whether the contents were still under pressure, was not necessarily an accurate evaluation of survival but did indicate a stage in

TABLE 3. Survival of *Heterodera glycines* at various moisture levels under greenhouse conditions.

Moisture conditions	Number of months survived in: Test 1 Sandy soil	Test 1 Clay soil	Test 2 Sandy soil
Flooded	7	19	11
Saturated	29	38	63
Watered daily	90[a]	90	40
Air dry	29	38	35

[a]Negative after 96 months.

the degeneration of the larvae. The bioassay was the most practical test in that it assessed the infectivity of the larvae; turgidity was a rough estimate of this capability.

Endo (2) reported that *H. glycines* larvae survived only 30 min at 100 F (37.8 C). At 40 C (104 F), larvae remained infective for 28 hr and appparently were alive somewhat longer in our tests (Table 1). Ichinohe (6) reported that eggs remained viable in cysts held at -40 C for as long as 7 months, and Slack and Hamblen (13) obtained larval emergence from cysts maintained at -24 C for 18 months. However, larvae are sensitive to freezing; infectivity failed to occur after 1 day when ice crystals formed in the water surrounding them, even though movement and turgidity were recorded for a longer period.

H. glycines survived longer in the soil than in water, but the effect of temperature presented a similar pattern. The fact that infection occurred in soil stored air dry at 40 C for 50 days whereas no larvae were recovered from a funnel may indicate that the presence of host roots had a stimulating effect on the near-dead larvae. On the other hand, the host may provide a hatching stimulus for eggs which would not otherwise hatch. Previous studies indicated that *H. glycines* did not develop in the host at 34 C and these studies showed that nemas survived at 36 C for 6-11 months (Slack et al., unpublished data). This indicates that the lack of development in host tissues at 34 C is not due to death of the nemas. A change in host physiology at 34 C might occur which prevents nema development or this temperature may induce a type of dormancy in the nemas. Infection occurred over a period of 30 months at 24 and 28 C, and in some field soils the temperature at a depth of 6 inches may not exceed 28 C. Since there are periods when the soil at this depth has a much lower temperature, this may help to account for the fact that *H. glycines* can survive in the field in the absence of a host for several years. The longer survival time at 24, 28, 32 and 36 C in dry soil as compared to moist soil was probably related to egg hatching. At the low moisture level the egg hatch would probably have been low or none. Larvae in eggs should survive longer than larvae free in the soil because they do not desiccate as readily in eggs.

Drying or flooding of the soil did not readily kill the nematodes, but soil type influenced the time of survival. The survival period was longer in a clay type soil similar to that on which rice might be grown. Unpublished field observations have indicated that the *H. glycines* population is not reduced appreciably during a rice crop, even though rice is not a host. Lack of egg hatching under low oxygen levels may account for the survival of *H. glycines* under flooded conditions in the field and greenhouse. In dry soil the nematodes survived for 3 years, which indicates they could survive in soil balls on implements, boots, shoes or other possible carriers to be dropped off in other fields. Under normal water conditions the nematodes were able to survive almost 8 years in the absence of a host, implying that very long rotations would be required to reduce the population to a minimum in the soil. Even in the absence of soybeans, certain common weeds are hosts and could serve to maintain a source of the nema for reestablishment of the population when planted to a susceptible crop.

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