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THE EFFECTS OF MOISTURE ON THE THERMOSENSITIVITY OF *GLOBODERA ROSTOCHIENSIS* (NEMATODA)[1]

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Abstract

The sensitivity of *Globodera rostochiensis* second-stage juveniles in eggs inside cysts (encysted J2) to heat was evaluated over a range of temperature and moisture levels. Encysted J2 in air dried cysts incubated in dry sand were less sensitive to thermal stress than those maintained under moist conditions. Heating of moist cysts to 50°C for 20 minutes in wet sand resulted in death of the encysted J2. Presoaking air dried cysts for 48 hours in water prior to heating in dry sand did not increase the sensitivity of encysted J2 to heat. Thermal sensitivity of encysted J2 presoaked for 48 hours in water or glycerine and then heated to 51°C or 56°C indicated that both water and glycerine conducted heat to the J2. Hydrated (water soaked) encysted J2 were killed at lower temperatures and at shorter exposures than were anhydrobiotic (glycerine soaked) encysted J2. Presoaking cysts in potato root diffusate to initate hatching did not increase the thermal sensitivity of encysted J2.

Compendio

Se evaluó la sensibilidad al calor de juveniles de segundo estado en huevos dentro de los quistes (J2 enquistados) bajo diversas niveles de temperatura y humedad. Los J2 equistados, en quistes secados al aire e incubados en arena seca, fueron menos sensibles al estrés al calor que aquellos mantenidos bajo condiciones húmedas. El calentamiento de quistes remojados a 50°C por 20 minutos en arena húmeda dio como resultado la muerte de los J2 enquistados. El remojo previo en agua por 48 horas de los quistes secos al aire antes del calentamiento en arena seca no incrementó la sensibilidad al calor de los J2 enquistados. La sensibilidad al calor de los J2 enquistados preremojados por 48 horas en agua o glicerina y luego calentados a 51°C o 56°C indicó que tanto el agua como la glicerina condujeron calor a los J2. Los J2 enquistados e hidratados (remojados en agua)

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fueron muertos a menores temperaturas y exposiciones más cortas que los no hidratados (remojados en glicerina). El remojo previo de los quistes dispersos en las raíces de papa para iniciar la eclosión no incrementó la termosensibilidad de los J2 enquistados.

Introduction

A number of workers have determined the length of time and the temperature required to kill juveniles in eggs inside nematode cysts (encysted J2). Exposure to 48°C for 30 minutes in a water bath completely eliminated encysted J2 of the potato strain of *Heterodera schachtii* (15). Encysted J2 of *Globodera rostochiensis* were killed when exposed for 40 minutes to 50°C in water but air dried encysted J2 survived 4 days at the same temperature (2). Encysted J2 in dry soil appear better able to withstand high temperatures than those in moist soil (6, 8). Subsequent hatch of *G. rostochiensis* and *G. pallida* was completely inhibited when dry cysts were exposed to 90°C but was not affected at lower temperatures (13). Hatch was reduced when wet cysts were exposed to 40-50°C with complete inhibition of hatch after 4 minutes at 50°C. Short exposures to 40°C tended to stimulate hatch, as more juveniles emerged from cysts exposed 1, 4, and 8 minutes than from untreated cysts. Brodie (1), showed that hatch of encysted J2 of *G. rostochiensis* was inversely related to length of cyst exposure to 44°C in water, with no hatch after 3.5 hours exposure.

Minimum time/temperature required to kill *H. schachtii, H. glycines* and *H. tabacum* J2 in wet cysts appear to be similar to that required to kill J2 of *Globodera* spp. (4, 9, 11, 12). Cysts apparently do not protect J2 inside eggs from temperature stress as time/temperature required to kill encysted J2 of *H. glycines* was no different than that for J2 in free eggs (4). Encysted J2 of *H. schachtii* collected from air-dry soil where mean minimum and maximum soil temperatures 7.5 cm deep ranged between 24-31°C and 30-42°C, respectively, were viable but their infectivity was decreased in the top 2.5 cm of soil (14). The insensitivity of J2 in eggs of air dried cysts to high temperatures appears to be an adaptation for survival in the upper few centimeters of soil. Both temperature, a function of cyst position in the soil profile, and moisture are implicated in this response. Our objective was to determine the influence of moisture on the sensitivity of *G. rostochiensis* to high temperature.

Materials and Methods

Ten air dried *Globodera rostochiensis* cysts of uniform size and age were added to 3 cc of water, sand, or a 2:1 mixture of soil and peat in 12 mm-d test tubes. Water was added or not to the sand and soil mixture to obtain 0, 15, 25, 35, and 100% (flooded) moisture by weight. Tubes were covered with parafilm and heated immediately or allowed to acclimate for 48 hours

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before heating. Treatments consisted of various lengths of time at 40, 45, 50 and 55°C in a Fischer Isotemp dry bath. Timing of treatments began when the contents of the tubes attained desired temperatures and ended when tubes were removed to cool to room temperature in a water bath. Each treatment was replicated 5 times.

Viability of second stage juveniles inside eggs in cysts (encysted J2) was determined both by hatch in potato root diffusate (PRD) for 3 weeks and by visual observation of J2 released from eggs by crushing cysts (4). Viable J2 were distinguished by the presence of a clearly delimited boundary between the esophagus and intestine with limited vacuolization of the intestine. Nonviable J2 were highly vacuolated with no distinct border between the esophagus and intestine.

Three factorial experiments were performed to determine if water predisposes encysted J2 to temperature stress or if its presence serves for heat conduction. Dry cysts of uniform size and age stored at 21°C and 45-48% relative humidity for 3-4 months and dry cysts presoaked in water 48 hours were added to sand with 35% water by weight or air dried sand in 12 mm-d tubes. Each tube with 10 cysts was then heated to 50°C for 10, 12, 15, and 20 minutes. Viability of encysted J2 was determined by hatch in PRD and by visual observations for 3 replications of each treatment. In a second experiment 10 dry cysts of uniform size and age were presoaked for 48 hours in 0.2 ml of water or glycerine. Those cysts in water contained eggs with hydrated J2 whereas those in glycerine contained eggs with anhydrobiotic J2. Cysts from each of these treatments were heated to 51°C and 56°C for various times in 3.0 ml water or glycerine. Afterwards, the cysts were exposed to PRD for 2 weeks and number of hatched J2 determined. There were 8 replications of each treatment.

In the third experiment, dry cysts of uniform size and age were presoaked for 48 hours in water or PRD and then heated to 45°C in water or PRD. Cysts were then crushed and their contents visually examined to determine J2 viability.

Results

Encysted *G. rostochiensis* J2 heated in water were killed after 2 hrs. exposure to 45°C, 5 minutes exposure to 50°C, and immediately when exposed to 55°C. Survival of encysted J2 heated in moist soil was not significantly different from survival in water. There were no differences ($P = 0.05$) between survival of encysted J2 heated in sand or soil. Encysted J2 in dry cysts heated in dry sand or soil survived the greatest time/temperature combination tested, 47 hrs. at 55°C. Heating encysted J2 to 40°C in water resulted in reduced viability after 5 days with complete elimination of the encysted J2 after 7 days (Figure 1).

Exposing dry and presoaked cysts (48 hrs. in water) for 10, 12, 15, and 20 minutes to 50°C indicated that survival of J2 inside wet and dry cysts

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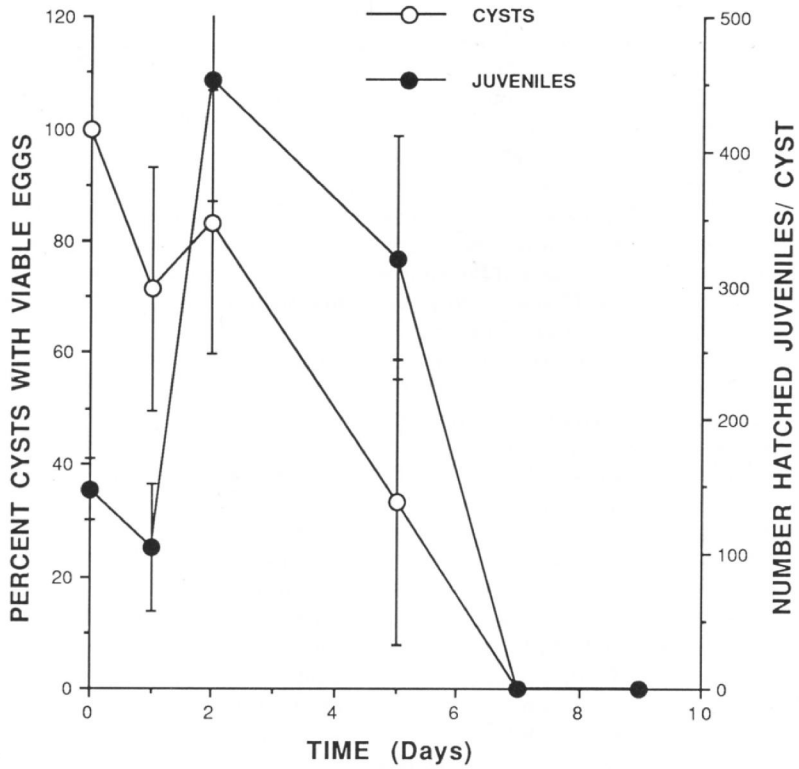

FIG. 1. Viability and hatch of encysted juveniles of *Globodera rostochiensis* heated to 40 C in water.

was significantly (P = 0.01) lower in wet soil; compared to survival in dry soil (Table 1). The survival of J2 inside wet and dry cysts in dry soil was not significantly affected.

Encysted J2 that were heated in glycerine survived up to 60 minutes at 51°C and 56°C; whereas encysted J2 were killed when exposed to these temperatures in water for only 10 minutes (Table 2). Thermal mortality, as measured by J2 hatch after heating, was greater (P = 0.05) in cysts presoaked in water than in those presoaked in glycerine at both 51°C and 56°C. Mortality was also greater (P = 0.001) in cysts that were heated in water than in cysts heated in glycerine at both 51°C and 56°C. Also presoaking in water reduced (P = 0.005) hatch when compared to presoaking in glycerine. Percent survival of encysted J2 presoaked and heated in water

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TABLE 1.—*Percent of* Globodera rostochiensis *cysts with viable eggs after various treatments and exposures to 50°C.*

Treatment	10 min.	12 min.	15 min.	20 min.	$\bar{x}$
Wet cysts - wet soil	0.0[1]	0.0	0.0	20.0	5.0
- dry soil	-	-	89.0	100.0	94.5
Dry cysts - wet soil	14.3	0.0	18.8	11.8	11.2
- dry soil	100.0	100.0	100.0	100.0	100.0

[1]Data analyzed after arc sin transformation.

Linear contrasts	Significance level
Wet soil treatments versus dry soil treatments	0.01
Wet cyst treatments versus dry cyst treatments	NS
wet cyst wet soil versus dry cysts wet soil	NS
Wet cysts dry soil versus dry cysts dry soil	NS

TABLE 2.—*Hatch of* Globodera rostochiensis *juveniles in potato root diffusate from cysts presoaked and heated to 51°C* and 56°C in water or glycerine for various times.

	Water presoak		Glycerine presoak	
Treatment[1]	Heated in water	Heated in glycerine	Heated in water	Heated in glycerine
51°C 10 min.	0	6.6	0	27.6
20 min.	0	5.9	0	70.4
40 min.	0	33.9	0	49.3
60 min.	0	11.3	0	48.8
$\bar{x}$	0.0	14.4	0	49.0
56°C 0 min.	0	3.4	0	74.3
10 min.	0	32.5	0	36.3
20 min.	0	11.4	0	39.5
40 min.	0	15.8	0	67.5
$\bar{x}$	0.0	15.8	0	54.4

[1]AOV: 51°C F = 17.77 MSE = 120.4 MSB = 118.5 df = 3, 9.
56°C F = 16.72 MSE = 157.63 MSB = 50.19 df = 3, 9.

Linear contrasts	Significance level	
	51 C	56 C
Presoaked in water versus soaked in glycerine	0.05	0.05
Heated in water versus heated in glycerine	0.001	0.001
Presoak water, heat in glycerine versus presoak glycerine heat in glycerine	0.005	0.005

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or PRD indicated no significant difference (P = 0.05) in survival at the exposure times tested (Figure 2).

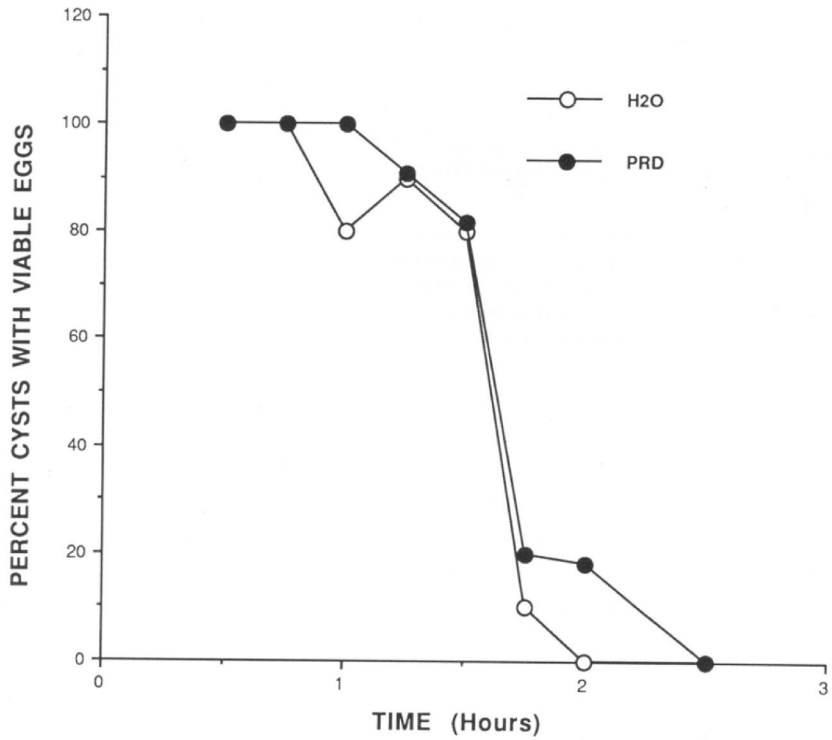

FIG. 2. Survival of *Globodera rostochiensis* at 45 C after presoaking and heating in either water or potato root diffusate (PRD).

Discussion

Clearly, the presence of water increases the sensitivity of encysted *G. rostochiensis* J2 to temperatures of 45°C to 50°C. In previous studies we found that the survival of *G. rostochiensis* in soil subjected to solar heat under plastic mulch is dependent on soil moisture (7). Water may have two effects on the sensitivity of *G. rostochiensis* encysted J2 to heat. First, the conduction of heat through water in soil is much more efficient than through pores filled with air. This is supported by the fact that encysted J2 in presoaked cysts heated in dry soil were less affected than were those in dry cysts heated in wet soil. Also the cyst wall apparently is not an effective barrier for

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protecting encysted J2 of *Heterodera glycines* from temperature stress when cysts are heated in water (4). In our study hydrated encysted J2 that were heated in air or in dry soil did not exhibit the same mortality as did those heated in wet soil.

Secondly, water may act to predispose encysted J2 to temperature stress. Encysted J2 of *G. rostochiensis* are in a state of incomplete hydration (3). When immersed in glycerine these J2 remain incompletely hydrated. Cysts filled with glycerine apparently conduct heat similar to those filled with water, but the anhydrobiotic J2 inside these cysts appear to have a greater resistance to temperature stress than do those in cysts filled with water. Survival of encysted J2 that were presoaked in water was significantly less than survival of those presoaked in glycerine; even when both were heated in glycerine, suggesting that water increases the sensitivity of encysted J2 to heat.

Encysted J2 of *G. rostochiensis* take up water when exposed to PRD (3). Response to the hatching factor in PRD is presumably rapid as only 5 minutes exposure results in increased hatching activity (5). Brief exposure to PRD increases the sensitivity of encysted *G. rostochiensis* J2 to desiccation (10). In our experiments, exposure of cysts to PRD for 48 hours to initiate the hatching sequence did not predispose encysted J2 to thermal stress. Apparently the increased water content of encysted J2 resulting from exposure to PRD for only 48 hrs. was not sufficient to increase their sensitivity to heat in our experiments.

The sensitivity of encysted J2 of *G. rostochiensis* may be an important factor in this nematode's inability to establish populations in hot tropical areas. This factor appears to be a weak link in the nematode's life cycle and could be important in the development of alternative controls of *G. rostochiensis* by combining solarization with other control tactics (7).

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