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Heterodera glycines Cyst Components and Surface Disinfestants Affect *H. glycines* Hatching[1]

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Abstract: We investigated the effects of *Heterodera glycines* cyst components and surface disinfestants on hatching of *H. glycines* eggs in vitro. Eggs were incubated in either *H. glycines* cyst wall fragments, cyst wall and egg rinsate, egg homogenate, or control solutions of soybean root diffusate, sterile distilled water, or zinc sulfate. Hatch in cyst wall and egg rinsate, and egg homogenate, was greater ($\alpha = 0.05$) than hatch in sterile distilled water; however, it was not different from hatch in zinc sulfate according to Dunnett's test. Hatch in cyst wall fragments was similar to hatch in sterile distilled water. To determine whether surface disinfestants affected hatch, eggs were treated first with chlorhexidine diacetate, mercuric chloride, sodium hypochlorite, or streptomycin sulfate and then incubated in *H. glycines* egg homogenate, soybean root diffusate, sterile distilled water, or zinc sulfate. Hatch of eggs treated with chlorhexidine diacetate, mercuric chloride, and streptomycin sulfate was reduced ($\alpha = 0.05$), and hatch of eggs treated with sodium hypochlorite was increased ($\alpha = 0.05$) relative to hatch of nontreated eggs in all incubation solutions except zinc sulfate according to Dunnett's Test. Hatch in zinc sulfate was similar among all surface disinfestants except mercuric chloride, where hatch was reduced relative to hatch of nontreated and other surface disinfestant-treated eggs.

Key words: chlorhexidine diacetate, cyst, egg hatch, hatch inhibitor, hatch stimulator, *Heterodera glycines,* mercuric chloride, sodium hypochlorite, soybean cyst nematode, soybean root diffusate, streptomycin sulfate, surface disinfestation.

The encysted eggs of the soybean cyst nematode, *Heterodera glycines* Ichinohe, remain viable and unhatched for up to 11 years (Inagaki and Tsutsumi, 1971). This delay in hatching, or diapause, is an effective survival strategy enabling the nematode to avoid environmental conditions unfavorable for hatch and infection. During diapause, hatch-inhibiting compounds within the cyst may suppress hatch of encysted *H. glycines* eggs. Okada (1972a) reported that a hatch inhibitor was washed from the cyst wall or egg surfaces. In contrast to cysts, egg homogenate stimulated hatch of *H. glycines* eggs (Okada, 1972b). A mixture of hatch-inhibiting and -stimulating compounds may regulate hatch in *H. glycines*.

Hatch processes in cyst nematodes have been summarized by Jones et al. (1998). Hatch-inhibiting compounds may interfere with the induction of hatch by blocking the ligand domains of receptors located on the eggshell, thereby preventing the receptor from recognizing hatch-stimulating compounds. Once hatch stimulation occurs, increased eggshell permeability results in the release of trehalose, hydration of the unhatched juvenile, and subsequent hatching of the nematode.

With the aim to better understand hatch biology, our ultimate goal was to isolate and identify hatch-inhibitory and -stimulatory compounds from *H. glycines* reported by Okada (1972a; 1972b). Therefore, our first objective was to determine if the presence of these compounds could be detected consistently.

Several reports indicate involvement of microbes in cyst-nematode hatching. Isolates of Rhizobacteria have been shown to stimulate hatch in *Globodera rostochiensis* and inhibit hatch in *H. schachtii* (Cronin et al., 1997; Sikora and Hoffmann-Hergarten, 1993). To lessen the influence of microbes on hatch, surface disinfestation of nematode eggs commonly has been used to reduce microbial contamination during in vitro laboratory hatch studies. Sodium hypochlorite has been used to collect and surface disinfest egg-mass eggs of *H. glycines* and *Meloidogyne* spp. (Hussey and Barker, 1973; Koenning and Barker, 1985; Thompson and Tylka, 1997). Mercuric chloride has been used to surface disinfest *H. zeae* cysts to obtain microbe-free eggs (Arjun et al., 1982) and streptomycin sulfate has been used to reduce microbial contamination of hatch solutions and to surface disinfest nematodes (Huettel, 1990; Mountain, 1955). Results of our preliminary experiments indicated that treatment of *H. glycines* eggs with 0.5% chlorhexidine diacetate (Acedo and Dropkin, 1982) reduced hatch. Consequently, our second objective was to determine if treatment of *H. glycines* eggs with surface disinfestants affected hatch.

Materials and Methods

Inoculum preparation: Soil infested with *H. glycines* was collected in May of 1998 and 1999 from a field near Ames, Iowa, in which soybean, *Glycine max* (L.) Merrill, had been grown annually since 1995. Soil was stored at approximately 25 °C, and cysts were extracted from soil within 9 months of collection.

Infested soil was suspended in water, and cysts were collected on a 250-µm-pore sieve by wet sieving followed with decantation (Gerdemann, 1955). Plant and soil debris were separated from cysts using sucrose-centrifugal flotation methods modified from Jenkins'

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description (1964). Cysts and debris were suspended in 30 ml of 1,362 g/liter sucrose solution and centrifuged at 880*g* for 2 minutes at 25 °C; then the supernatant was poured through a 250-µm-pore sieve to collect cysts. Additional debris was removed from cysts by overlaying cysts and debris on 30 ml of 1,362 g/liter sucrose and centrifuging at 4,000*g* for 1 hour at 4 °C. Cysts were recovered from the supernatant as described above. Eggs were released from cysts with a stainless-steel pestle attached to a variable-speed stirrer (T-line Lab Stirrer; Talboys Engineering Corp., Montrose, PA) as described previously by Niblack et al. (1993). Debris was removed from the eggs by centrifuging for 1 minute at 880*g* in 454 g/liter sucrose (Jenkins, 1964), after which the supernatant was poured through a 30-µm-pore sieve to recover the eggs. Eggs were rinsed three times with and suspended subsequently in sterile distilled water and then stored at 4 °C for no more than 48 hours before use in experiments.

Laboratory hatch assay procedures: Hatch of *H. glycines* eggs was assessed in three sets of experiments using the following hatch assay procedures. For each experiment, 300 µl of incubation solutions were dispensed into randomly assigned wells of sterile, 48-well tissue culture trays (Becton Dickinson and Company, Franklin Lakes, NJ). Each tray represented a replication or block in a randomized complete block design in all three sets of experiments. At the beginning of each experiment, incubation solutions were allocated to all trays needed for the study, and the trays were sealed with parafilm (American National Can, Chicago, IL) and stored at –20 °C until thawed at 25 °C for use.

Adapting methods described by Wong et al. (1993), sieves were constructed of 30-µm-pore nylon mesh (Tetko, Briarcliff Manor, NY) held between 1.0-cm-diam. × 1.5-cm-high and 0.7-cm-diam. × 1.5-cm-high cylinders that were cut from 1.0- and 0.1-ml pipet tips (Fisher Scientific, Pittsburgh, PA), respectively. Approximately 2,000 eggs were decanted onto each sieve, and sieves were placed into wells containing incubation solutions. Eggs were incubated in incubation solutions at 25 °C in darkness. Sieves were transferred to duplicate tissue culture trays containing incubation solutions every 2 days for a total of 18 days, and hatched juveniles remaining in the wells were counted for each sampling day. At the end of each experiment, the initial number of eggs on each sieve was determined by summing the number of unhatched eggs remaining on the sieve with the number of juveniles that had hatched during the experiment. The sum of hatched juveniles over the 18-day incubation period was divided by the initial number of eggs and then multiplied by 100 to calculate the percentage cumulative hatch (Wong et al., 1993).

Effect of cyst components on hatching: Experiments were conducted to determine whether components of the *H. glycines* cyst affected hatching of the eggs. Cysts were separated into three components: cyst wall fragments, cyst and intact egg rinsate, and egg homogenate. Intact cysts, obtained as described above, were separated manually into groups of 864 cysts; each group represented a replication. Cyst walls were broken and eggs were freed from cysts using a 1.5-ml microcentrifuge tube and pellet pestle (Kontes Scientific Glassware/Instruments, Vineland, NJ). One milliliter of sterile distilled water was added to the broken cysts and freed eggs, the suspension was centrifuged at 6,000*g* for 1 minute at 25 °C, and rinsate was decanted and saved. This process was repeated five additional times. The decanted cyst and egg rinsate was collected from each batch of cysts and diluted to a volume of 7.5 ml with sterile distilled water.

The broken cyst walls were separated from intact eggs using nested 150-, 75-, and 30-µm-pore nylon-mesh sieves, which were made using a method modified from Wong et al. (1993). Nylon mesh (Tetko, Briarcliff Manor, NY) was held together between 18-mm-diam. × 20-mm-high and 20-mm-diam. × 20-mm-high polypropylene test-tube caps (Fisher Scientific, Hanover Park, IL) with the enclosed ends removed. Cyst walls were collected on the 150-µm-pore sieve, and intact eggs were collected on the 30-µm-pore sieve. Material collected on the 75-µm-pore sieve was discarded. Broken cyst walls and intact eggs were washed with 50 ml of sterile distilled water while on the sieves. Broken cyst walls and intact eggs were transferred with tap water to individual 1.5-ml microcentrifuge tubes. Cyst walls were centrifuged at 6,000*g* for 1 minute in 1,362 g/liter sucrose to separate any intact eggs and juveniles from cyst walls. Subsequently, the sucrose solution containing eggs and juveniles was discarded, and the pellet of broken cyst walls remained in the tube. Cyst walls were rinsed three times with sterile distilled water by centrifuging at 6,000*g* for 1 minute, followed by decantation. Cyst walls and intact eggs were concentrated into 0.1-ml volumes and sonicated at 20 kHz for 7 seconds using a 2-mm-diam. probe (High Intensity Ultrasonic Processor, 50-Watt 4710 Series; Cole-Parmer-Instrument Co., Chicago, IL), and then microcentrifuge tubes containing the components were placed on ice for 30 seconds. Sonication and cooling were repeated five additional times to produce cyst wall fragments and egg homogenate. Cyst wall fragments and egg homogenate were diluted to a volume of 6.5 and 7.5 ml, respectively, with sterile distilled water.

To remove intact juveniles and eggs from egg homogenate and cyst wall and egg rinsate, each batch of these materials was sterilized individually by passing the solutions through a 0.22-µm-pore cellulose-acetate vacuum filter (Corning Costar, Corning, NY) that resulted in a final volume of 6.5 ml. The three cyst components (cyst wall fragments, cyst and intact egg rinsate, and egg homogenate) were diluted sequentially from an equivalent of 133 to 66 and 17 cysts/ml with sterile

distilled water. The three cyst components were kept on ice or at 4 °C during preparation.

To assess hatch, washed, free eggs, obtained from the nematode population that was used to recover the cyst components, were rinsed in sterile distilled water and dispensed onto sieves as described above. The sieves were placed in suspensions of cyst components, sterile distilled water, 3 mM zinc sulfate (Fisher Scientific, Pittsburgh, PA), or soybean root diffusate.

Soybean root diffusate was prepared, using a combination of two methods reported previously (Levene et al., 1998; Tefft and Bone, 1985), from *G. max* cv. Corsoy 79 plants grown in a two parts soil:one part sand medium in a greenhouse from June to October 1998. Five weeks after planting, the soybean plants were removed from the soil:sand medium, and roots were rinsed with tap water to remove soil and sand particles. The roots of six intact plants were incubated with 400 ml of distilled water in a 500-ml Erlenmeyer flask covered with aluminum foil. Plants were incubated for 48 hours in these flasks in the greenhouse under natural light conditions, with an average daytime temperature of 20 °C to 30 °C. The volume of diffusate was collected and recorded. The roots were excised at the hypocotyl, blot-dried, and weighed. The concentration of root diffusate was quantified as root-gram-hour (RGH), which was calculated as root mass (g) multiplied by incubation time (hour) and then divided by the amount of diffusate obtained (ml) (Tefft and Bone, 1985). After the diffusate was centrifuged at 138,000*g* for 45 minutes at 4 °C, the pellet was discarded and supernatant was sterilized by passing it through a 0.22-µm-pore cellulose-acetate filter (Corning Costar, Corning, NY). Sterile diffusate was stored at −20 °C and diluted to a concentration of 2.0 RGH with sterile distilled water immediately before use in the experiments.

The experiment was conducted four times, twice each with cysts and eggs collected in 1998 and 1999, within 4 to 18 weeks after soil collection. Treatments were replicated four times within each experiment, but the cyst wall fragment component was tested in only three of the four experiments. Percent cumulative hatch after 18 days of incubation was calculated for each treatment as described above.

Effect of chlorhexidine diacetate on hatching: A two-by-eight complete factorial experiment was conducted to investigate the effects of treating eggs with chlorhexidine diacetate on hatching. Eggs were extracted from cysts obtained from soil collected in 1998. Eggs were incubated for 15 minutes at 25 °C in 0.5% chlorhexidine diacetate (Sigma Chemical Company, St. Louis, MO) or treated with a comparable volume of sterile distilled water as a control treatment. Then, eggs were concentrated into a pellet by centrifuging at 880*g* for 4 minutes at 25 °C. After the supernatant was decanted, eggs were rinsed twice with sterile distilled water and then incubated in sterile distilled water, 3 mM zinc sulfate, one of three concentrations of soybean root diffusate (0.5, 1.0, or 2.0 RGH) as described above, or one of three concentrations of egg homogenate as described below.

Egg homogenate solution was prepared by sonicating four batches of 70,000 eggs as described above with each batch assigned to a replication or block. Sterile distilled water was added to each batch of egg homogenate to give a final volume of 15 ml. Each batch of egg homogenate was individually sterilized by vacuum filtration with a 0.22-µm-pore filter. The egg homogenate was diluted sequentially with sterile distilled water from an equivalent of 5,000 to 2,500 and 625 egg equivalent/ml. Eggs and egg homogenates were kept on ice or at 4 °C during the egg homogenate preparation process.

The experiment was conducted four times, and eggs used in these experiments were extracted 12, 16, 20, and 32 weeks after soil collection. All treatment combinations were replicated a total of 14 times over the course of the four experiments. Percentage cumulative hatch after 18 days of incubation was calculated as described above.

Effect of various surface disinfestants on hatching: To investigate the effects of various surface disinfestants on *H. glycines* egg hatching, a five-by-eight complete factorial experiment was conducted. Eggs were extracted from cysts obtained from infested soil collected in 1999. Eggs were incubated at 25 °C for 15 minutes in sterile distilled water, 0.5% chlorhexidine diacetate (Acedo and Dropkin, 1982), and 0.1% streptomycin sulfate (Mountain, 1955), for 2 minutes in 0.5% sodium hypochlorite (Thompson and Tylka, 1997), or for 1 minute in 0.02% mercuric chloride (Arjun et al., 1982). Subsequently, eggs were concentrated into pellets by centrifuging at 6,000*g* for 1 minute, supernatants were decanted, and eggs were rinsed twice with sterile distilled water. Eggs then were incubated in either sterile distilled water, 3 mM zinc sulfate, one of three concentrations of soybean root diffusate (0.5, 2.0, or 4.0 RGH), or one of three concentrations of egg homogenate (625, 2,500, or 5,000 egg equivalent/ml). The soybean root diffusate and egg homogenate were prepared as described above.

The experiment was conducted twice with eggs extracted 17 and 24 weeks after soil collection. Treatments were replicated four times in each experiment, and percentage cumulative hatch was calculated after 18 days of incubation as described above.

Data analysis: In all experiments, percentage cumulative hatch data were transformed to natural log. Results from repeated experiments were similar according to analysis of variance (ANOVA) and subsequently combined for analysis. Transformed data were analyzed by ANOVA using a mixed linear model via Proc MIXED command in Statistical Analysis System (SAS) version 6.12 (SAS Institute Inc., Cary, NC) (Littell et al., 1996). A one-way ANOVA was conducted on the data from the

experiment investigating the effect of cyst components on hatch, whereas a two-way ANOVA was conducted for experiments investigating the effects of surface disinfestation on hatch. For all analyses, effects of experiment, replication, replications within experiment, and replication by factor (incubation solution or surface disinfestant) interactions were designated random variables. Surface disinfestation experiments were analyzed for main and interaction effects. When significant ($P \leq 0.05$) main and interaction effects were detected by ANOVA, treatment means were compared to control incubation solutions by performing the Dunnett's test (Steele and Torrie, 1980) with SAS.

To determine whether hatch was different from control incubation solutions, hatch in the incubation solutions was compared to hatch in sterile distilled water (no hatch stimulation) and zinc sulfate (artificial hatch stimulation) of similarly treated eggs (i.e., within surface-disinfestant treatment) using Dunnett's test ($\alpha = 0.05$) on transformed data. To determine the effect of surface disinfestant on hatching, hatch of surface disinfested-treated eggs was compared to hatch of untreated eggs within an incubation solution using Dunnett's test ($\alpha = 0.05$) on the transformed data.

Results

Effect of cyst components on hatching: Cumulative hatch after 18 days ranged from 7% to 35% for individual treatments (Table 1). Hatch of eggs in the two greatest concentrations of cyst wall and egg rinsate and egg homogenate was 2.0 to 3.8 times greater ($\alpha = 0.05$), respectively, than hatch in sterile distilled water and not different from hatch in zinc sulfate. Hatch in sterile distilled water was not different from hatch in soybean root diffusate and the remaining cyst components.

Effect of chlorhexidine diacetate on hatching: Surface disinfestation with chlorhexidine diacetate decreased ($P \leq 0.05$) percent egg hatch by 50% (Table 2). Hatch in zinc sulfate across surface-disinfestant treatments was 12 times greater ($\alpha = 0.05$) than hatch in sterile distilled water (Table 2). Hatching of eggs incubated in soybean root diffusate and in the lowest concentration of egg homogenate was not different from hatch in sterile distilled water. However, hatch in the two greatest concentrations of egg homogenate was more than double ($\alpha = 0.05$) hatch in sterile distilled water, although less ($\alpha = 0.05$) than hatch in zinc sulfate.

An interaction ($P \leq 0.05$) was detected between incubation solution and surface disinfestant (Table 2). Hatch of eggs treated with chlorhexidine diacetate was reduced ($\alpha = 0.05$) by 66% to 83% relative to hatch of untreated eggs in all incubation solutions except zinc sulfate, in which hatch of untreated and chlorhexidine diacetate-treated eggs was similar. Hatch in the greatest concentration of egg homogenate was 0.3 times greater ($\alpha = 0.05$) than hatch in sterile distilled water for both untreated and chlorhexidine diacetate-treated eggs; however, hatch was 60% and 80% less ($\alpha = 0.05$) than hatch in zinc sulfate, respectively.

Effect of various surface disinfestants on hatching: Overall, disinfestations with chlorhexidine diacetate, mercuric chloride, and streptomycin sulfate decreased ($P \leq 0.05$) hatch, whereas sodium hypochlorite increased ($P \leq 0.05$) hatch (Table 3). The hatch in zinc sulfate was 6.0 times greater ($\alpha = 0.05$) than hatch in sterile distilled water.

Table 1. Effect of cyst components and other incubation solutions on hatch of *Heterodera glycines* eggs for 18 days relative to hatch in sterile distilled water and zinc sulfate.

Incubation solution	Cumulative hatch (%)
Sterile distilled water	7.2b
3 mM zinc sulfate	33.0a
Soybean root diffusate	8.6b
Cyst wall fragments (cysts/ml)	
17	10.1b
66	10.8b
133	11.4b
Cyst wall and egg rinsate (cysts/ml)	
17	11.4b
66	21.7a
133	22.0a
Egg homogenate (cysts/ml)	
17	14.4b
66	34.9a
133	24.8a

Values are means of 16 replications combined from four experiments, except for cyst wall fragment where data values are means of 12 replications from three experiments. Data were transformed to $\ln(x)$ for statistical analyses. Non-transformed data are presented. Means followed by (a) are different from sterile distilled water and by (b) are different from zinc sulfate according to Dunnett's test ($\alpha = 0.05$).

Table 2. Effects of incubation solution and surface disinfestation with chlorhexidine diacetate on hatch of *Heterodera glycines* eggs for 18 days.

Incubation solution	Untreated	0.5% Chlorhexidine diacetate	Main effect means
	Cumulative hatch (%)		
Sterile distilled water	5.4b	1.8b†	3.6b
3 mM zinc sulfate	45.2a	42.4a	43.8a
Soybean root diffusate (root-gram-hour)			
0.5	9.9b	1.7b†	5.8b
1.0	7.5b	2.2b†	4.9b
2.0	4.5b	1.2b†	2.9b
Egg homogenate (egg equivalent/ml)			
625	7.6b	1.7b†	4.7b
2,500	15.0ab	4.9b†	10.0ab
5,000	17.9ab	6.1ab†	12.0ab
Main effect means	14.1	7.8†	

Values are means of 14 replications combined from four experiments. Data were transformed to $\ln(x)$ for statistical analyses. Non-transformed data are presented. Individual treatment combination means within surface disinfestant followed by (a) are different from sterile distilled water and by (b) are different from zinc sulfate according to Dunnett's test ($\alpha = 0.05$). Within incubation solution means followed by (†) are different from untreated eggs according to Dunnett's test ($\alpha = 0.05$).

TABLE 3. Effect of incubation solution and surface disinfestant on hatching of *Heterodera glycines* eggs for 18 days.

Incubation solution	Untreated	0.5% Chlorhexidine diacetate	0.02% Mercuric chloride	0.5% Sodium hypochlorite	0.1% Streptomycin sulfate	Main effect means
	Cumulative hatch (%)					
Sterile distilled water	7.2b	1.5b†	1.3†	11.4b	1.9b†	4.7b
3 mM zinc sulfate	34.9a	28.7a	15.2a†	39.8a	38.3a	31.4a
Soybean root diffusate (root-gram-hour)						
0.5	8.4b	0.7b†	1.6†	19.2b	3.2b†	6.6b
2.0	5.4b	0.8b†	0.9†	9.3b	7.6ab	4.8b
4.0	2.3ab	1.7b†	1.4	8.7b†	1.8b	3.2b
Egg homogenate (egg equivalent/ml)						
625	7.9b	2.6b†	1.4†	16.9b	3.8b†	6.5b
2,500	17.1ab	4.0b†	9.2a†	28.1b	8.7ab	13.4ab
5,000	19.1a	3.7b†	2.0†	23.4b	21.9a	14.0ab
Main effect means	12.8	5.5†	4.1†	19.6†	10.9†	

Values are means of eight replications combined from two experiments. Data were transformed to $\ln(x)$ for statistical analyses. Non-transformed data are presented. Individual treatment combination means within surface disinfestant followed by (a) are different from sterile distilled water and by (b) are different from zinc sulfate according to Dunnett's test ($\alpha = 0.05$). Within incubation solution means followed by (†) are different from untreated eggs according to Dunnett's test ($\alpha = 0.05$).

The hatch in the two greatest concentrations of egg homogenate was more than double ($\alpha = 0.05$) the hatch in sterile distilled water, although only half ($\alpha = 0.05$) of the hatch in zinc sulfate. Hatch in all three concentrations of soybean root diffusate and the lowest concentration of egg homogenate was similar to hatch in sterile distilled water.

An interaction ($P \leq 0.05$) was detected between incubation solution and surface disinfestant (Table 3). Hatch of eggs treated with chlorhexidine diacetate, mercuric chloride, or streptomycin sulfate and incubated in sterile distilled water, soybean root diffusate, and egg homogenate was 26% to 92% less ($\alpha = 0.05$) than that of untreated eggs. However, hatch of eggs treated with sodium hypochlorite was 0.2 to 2.8 times greater than hatch of untreated eggs in similar solutions, but this difference was significant ($\alpha = 0.05$) only for the greatest concentration of soybean root diffusate. Hatch in zinc sulfate was greater ($\alpha = 0.05$) than hatch in sterile distilled water and similar among untreated and surface disinfestant-treated eggs, except for mercuric chloride-treated eggs in which hatch was 56% less ($\alpha = 0.05$) than hatch of untreated eggs. Hatch in the two highest concentrations of egg homogenate was similar within untreated and streptomycin sulfate-treated eggs, which ranged from 1.4 to 11 times greater ($\alpha = 0.05$) than hatch of similar eggs in sterile distilled water. In addition, hatch in the greatest concentration of egg homogenate was similar to hatch in zinc sulfate within untreated and streptomycin sulfate-treated eggs.

DISCUSSION

In contrast to Okada's reported results (1972a), *H. glycines* cyst wall and egg rinsate stimulated, rather than inhibited, hatch in our experiments. Okada detected hatch inhibition from cyst wall and egg rinsate of *H. glycines* cysts collected in autumn. In our work, cysts were collected in the spring. So a possible explanation for the discrepancy in hatch between the two studies is that hatch inhibitors may be present in the autumn but absent in the spring. Hatch inhibitors may have degraded while cysts remained in the soil over winter. Alternatively, if hatch inhibitors were present in our experiments, the eggs may not have responded to the concentrations tested or were not sensitive physiologically to the compounds. Inhibition of hatch in the autumn may prepare eggs for over-wintering in the soil, and attenuation of hatch inhibition may be involved in promoting hatch in the spring.

We confirmed Okada's report (1972b) of hatch stimulation by *H. glycines* egg homogenate. However, we observed one-third of the hatch stimulation observed by Okada at concentrations that were 12 to 24 times greater than those tested by Okada. These differences in hatch stimulation simply may reflect differences in egg homogenate concentrations tested. An additional source of variability between the studies may be due to the variation in the number of eggs contained within cysts. Okada (1972b) based egg homogenate concentrations using numbers of cysts rather than eggs, and the number of eggs per cyst may differ among experiments, which makes it difficult to compare concentration of egg homogenate between Okada's and our experiments.

Differences in hatch that we observed among the surface disinfestant treatments may be explained by their mode of action on other organisms. We observed reduced hatching of eggs treated with mercuric chloride. Mercury inactivates proteins by binding to thiol groups (Krushner, 1971). Inactivation of proteins important to metabolism would result in nematode death and sub-

sequent reduction in hatch. Streptomycin sulfate inactivates prokaryotic ribosomal proteins (Brock and Madigan 1988; Sande and Mandell, 1985), suggesting that it may not affect proteins involved in hatch of eukaryotes such as *H. glycines*. This mode of action may explain why streptomycin sulfate did not interfere with hatch stimulation by zinc sulfate and egg homogenate, but it is unclear why it reduced hatch in other incubation solutions.

Chlorhexidine diacetate interferes with adenosine triphosphatase (ATPase) activity in bacteria (Longworth, 1971). ATPases are important enzymes involved in many energy-requiring physiological pathways, some of which are conserved between prokaryotic and eukaryotic organisms (Alberts et al., 1994). Chlorhexidine diacetate may interfere with ATPase activity in unhatched eggs of *H. glycines,* which subsequently disrupts hatch or reduces the viability of unhatched juveniles. This hypothesis, however, does not explain why hatch in zinc sulfate was not altered after eggs were treated with chlorhexidine diacetate.

Treatment of eggs with chlorhexidine diacetate, sodium hypochlorite, or streptomycin sulfate did not alter hatch in zinc sulfate, which may indicate that zinc sulfate may affect hatch by a different mechanism from natural hatch stimulators. Rather than stimulating hatch physiologically, zinc sulfate may change the physical properties of the eggshell. Treatment of *M. naasi* eggs with sodium hypochlorite increases hatching (Ogunfowora and Evans, 1976), which may result from the destruction of the lipoprotein membrane of the eggshell. Because we observed increased hatch after treatment of *H. glycines* eggs with sodium hypochlorite and zinc sulfate, it is possible that zinc sulfate may alter the *H. glycines* eggshell in a similar manner. However, neither the presence of the lipoprotein layer in the eggshell nor its importance in hatch has been firmly established for *H. glycines* (Perry and Trett, 1986). Further investigation of the viability and developmental maturity of juveniles hatched in zinc sulfate may reveal clues on the role of this compound in *H. glycines* hatching.

Our observations of variable hatch stimulation for eggs treated with different surface disinfestants, and then incubated in egg homogenate, suggest a microbial role in hatch stimulation by *H. glycines* egg homogenate. Fungi or bacteria may metabolize the egg homogenate, resulting in the formation of compounds that stimulate hatch. An alternative explanation for this variable impact on hatch is that the surface disinfestants interfere with the eggs' ability to be stimulated by the egg homogenate. One example of this may be the removal or inactivation of protein receptors located on the surface of the eggshell that recognize compounds that elicit a hatch response. Further investigation will be required to clarify mechanisms of hatch stimulation by *H. glycines* egg homogenate.

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