Journal of Nematology 27(2):164–171. 1995.

Effects of Zinc Fertilization of Corn on Hatching of *Heterodera glycines* in Soil[1]

J. E. Behm,[2] G. L. Tylka,[2] T. L. Niblack,[3] W. J. Wiebold,[3] and P. A. Donald[3]

Abstract: Experiments were conducted to determine the effects of zinc fertilizers on hatching and soil population densities of *Heterodera glycines*. In vitro egg hatching in solutions of reagent-grade zinc sulfate and zinc chloride and fertilizer-grade zinc sulfate was significantly greater than hatching in deionized water, whereas zinc chelate fertilizer significantly inhibited egg hatching relative to deionized water. In greenhouse experiments, no differences in cumulative percentage egg hatch were detected in soil naturally infested with *H. glycines* amended with fertilizer-grade zinc sulfate and zinc chelate at rates equivalent to 0, 1.12, 11.2, and 112 kg Zn/ha and subsequently planted with corn (*Zea mays* L.). In a field experiment, no significant differences in *H. glycines* egg population densities and corn yields were detected among plots fertilized with 0, 11.2, and 22.4 kg Zn/ha rates of zinc chelate. Yields of *H. glycines*-susceptible soybean planted in plots 1 year after zinc fertilization of corn plots also were not significantly affected. Zinc compounds significantly affected *H. glycines* egg hatching in vitro, but had no effect on hatching in natural soils.

Key words: Glycine max, Heterodera glycines, hatching, nematode, soybean cyst nematode, zinc fertilizer.

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is a major pathogen of soybean, *Glycine max* (L.) Merrill, and is known to infest soil in most soybean-production areas worldwide (9). First discovered in the United States in North Carolina in 1954 (17), *H. glycines* has since become the most damaging soybean pathogen in the United States (6,12).

Rotation to nonhost crops is one of the most commonly employed tactics for *H. glycines* management in infested fields. As obligate parasites, hatched second-stage juveniles (J2) that fail to locate a susceptible host die without completion of their life cycle. Corn (*Zea mays* L.) is the most commonly grown nonhost crop in the Midwest, but 1 year of corn in the corn-soybean rotation typically used in much of the Midwest often has been ineffective at reducing *H. glycines* soil population densities substantially (T. Niblack, G. Tylka, unpub. data).

Zinc cations, in solution, can stimulate the in vitro hatching of several cyst nematode species, including *H. glycines* (4,13). However, there are no published reports of zinc-stimulated hatching under field conditions. Stimulated hatching of *H. glycines* by zinc when a nonhost crop is grown would be advantageous for managing *H. glycines* in infested fields by decreasing egg population densities. Zinc fertilizer also may increase corn grain yield (2,8,11,16), which would make this management tactic economically attractive for growers. The purpose of our research was to investigate the effects of zinc fertilization of corn on soil population densities of *H. glycines*.

Materials and Methods

In vitro experiments: Solutions of 3.0 mM Zn were made by dissolving 1.61 g reagent-grade zinc sulfate (22.74% zinc), 0.77 g reagent-grade zinc chloride (47.97% zinc), 2.62 g zinc chelate fertilizer (Sequestrene, 14.14% zinc, Ciba-Geigy, Greensboro, NC), or 1.19 g zinc sulfate fertilizer (31 Plus Zinc Sulfate, 31.00% zinc, Cozinco Sales, Denver, CO) each in 1 liter of deionized water previously adjusted to pH 7 with 1.0 M HCl and 1.0 M KOH. Also included was a deionized water control treatment with pH 7. *Heterodera glycines* race 3 was cultured on 'Corsoy 79'

Received for publication 14 September 1994.

[1] Journal Paper No. J-15977 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA 50011, Project 2285. Research supported in part by state and Hatch funds.

[2] Department of Plant Pathology, Iowa State University, Ames, IA 50011.

[3] Plant Sciences Unit, University of Missouri, Columbia, MO 65211.

The authors thank Amy T. S. Wong for technical assistance.

soybean in greenhouse pots, and eggs were obtained by dislodging females and cysts from roots of 28-day-old plants with a stream of water. Females and cysts were recovered on a 250-μm-pore sieve nested below a 850-μm-pore sieve and separated from root and soil debris by centrifugal flotation (7). Eggs were released from the females and cysts by crushing suspensions of the nematodes in water with a motorized pestle (10) and were collected on a 25-μm-pore sieve nested under a 75-μm-pore sieve. The eggs were surface disinfested in 0.5% chlorhexidine diacetate (hibitane) solution (1) for 15 minutes.

Microsieves were constructed of 38-μm-pore Nitex (Tetko, Lancaster, NY) monofilament screen suspended between 18-mm-d and 20-mm-d cylinders made from polypropylene test tube caps with the ends removed (15). Approximately 6,500 surface-disinfested eggs were placed into each microsieve and then placed in a hatching tray containing 12 ml of a treatment solution. Hatching trays were arranged in 20-cm-wide × 27-cm-long × 9.5-cm-deep polystyrene boxes in a randomized complete block design with five replications. The boxes were incubated in the dark at 25 C ± 2 C. Microsieves were transferred to new hatching trays with fresh treatment solutions every 2 days for 30 days. Hatched J2 were counted by direct microscopic observation. At the end of the experiment, the eggs and J2 remaining in the microsieves were counted, and the numbers of unhatched eggs were added to the accumulated totals of hatched J2 to determine the total numbers of eggs that had been originally placed in the microsieves. Total numbers of eggs in the microsieves were used to convert J2 numbers to cumulative percentage hatch values for all treatments at each sampling date. Percentage egg hatch data were subjected to analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test if significant ($P < 0.05$) treatment effects were detected (5). The statistical software PC/SAS (SAS Institute, Cary, NC) was used for all statistical analyses. This experiment was conducted twice.

Greenhouse experiments: Thirty-five 25-cm-d, 23-cm-deep, 11-liter-capacity plastic pots were filled with soil collected from a field infested with *H. glycines* race 1. The soil was a Webster clay loam (33.8% sand, 32.8% silt, 33.4% clay, 4.3% organic matter, pH 6.0, 27.6 meq cation exchange capacity) with 5.4 μg/ml diethylenetriaminepentaacetic acid (DTPA)-extractable Zn. Zinc sulfate or zinc chelate fertilizer was thoroughly mixed into each pot in amounts equivalent to rates of 1.12, 11.2, or 112 kg Zn/ha. The zinc fertilizer treatments and an untreated control (seven treatments) were assigned to individual pots in a randomized complete block design with five replications. Four kernels of 'LH119xLH51' corn were planted in each pot and thinned to one plant per pot 7 days later. Soil samples, consisting of five 2.5-cm-d, 20-cm-deep cores, were collected from each pot before fertilizer application and at 7, 14, 21, 28, and 60 days after planting. *Heterodera glycines* cysts and J2 were separated from 100-cm^3 aliquants of soil by elutriation (3) and were recovered on 250-μm-pore and 38-μm-pore sieves, respectively. The J2 were separated from sediments by centrifugal flotation (7) and counted. Eggs were extracted from cysts with a motorized pestle, recovered on a 25-μm-pore sieve, and stained with acid fuchsin (10) to facilitate counting. Egg and J2 densities were used to calculate cumulative percentage egg hatch for each sampling date and reproductive factors (Rf = final egg and J2 densities/initial egg and J2 densities) for each pot. Soil samples collected before and 7, 28, and 60 days after fertilizer application were analyzed for DTPA-extractable zinc by Belmond Labs, Belmond, Iowa. Data were subjected to a two-way ANOVA, followed by single-degree-of-freedom comparisons where appropriate (5). The experiment was conducted twice.

Field experiments: In 1992 and 1993, field plots were established on the Bradford Re-

search Center near Columbia, Missouri. Plots were 7.6 m long and four rows wide; row width was 75 cm. Soil type in both years was a Mexico silt loam (9% sand, 69.8% silt, 8.6% clay, 2.4% organic matter, pH 7.0, 21.2 meq cation exchange capacity), and the soil contained a concentration of 0.3 to 0.4 μg/ml DTPA-extractable Zn. The experimental design was a randomized complete block with five replications per treatment. Treatments were 0, 11.2, and 22.4 kg Zn/ha rates of zinc chelate fertilizer. The zinc fertilizer (Claw-El, 9.0% zinc, Brandt Chemical, Pleasant Plains, IL) was applied to appropriate plots by injecting solutions of zinc chelate dissolved in 16 liters of tap water into the area where the previous crop row had been planted; applicator chisel spacing was 75 cm. In both years, the previous crop was *H. glycines*-susceptible 'Williams 82' soybean.

No more than 2 days after application of zinc, corn ('Pioneer 3379') was planted at a rate of 57,600 kernels/ha without additional tillage. Planting dates were 1 June 1992 and 25 May 1993. Weed control was accomplished with a preplant application of glyphosate (1.8 kg a.i./ha) and a preemergence application of atrazine (1.35 kg a.i./ha) and metolachlor (2.24 kg a.i./ha). Before planting, 224 kg/ha N was applied as top-dressed ammonium nitrate; no phosphorus or potassium was applied.

Soil samples, consisting of 10 2.5-cm-d, 20-cm-deep soil cores, were collected from the center two rows of each plot 7 days after planting and harvest. Soil cores were combined and mixed, *H. glycines* cysts were extracted from 100-cm^3 subsamples of each sample, and eggs were extracted from cysts and counted as described for the greenhouse experiments. At maturity, plots were end-trimmed to 6.1 m and the middle two rows of each four-row plot were mechanically harvested. Corn yield was adjusted to 15% moisture.

To determine whether zinc fertilization of corn plots infested with *H. glycines* affected yield of a subsequent soybean crop, the *H. glycines*-susceptible soybean cultivar 'Pioneer 9391' was planted on 24 May 1993 without tillage into the previous corn rows within each corn plot used in 1992. Seeding rate was 360,000 seeds/ha. No nitrogen, phosphorus, or potassium fertilizer was applied. The plots were end-trimmed to 6.1 m long, and the middle two rows of each four-row plot were mechanically harvested at maturity. Soybean yields were adjusted to 13% moisture.

Data were subjected to ANOVA, and zinc effects were evaluated with single-degree-of-freedom contrasts (5). Yield data were analyzed untransformed, but *H. glycines* egg densities were transformed to $\log_{10}(n + 1)$ before analyses.

Results

Results were similar for both repetitions of the in vitro and greenhouse hatch experiments. Data from one repetition of each experiment are presented.

In vitro experiments: Most of the hatching in the deionized water and zinc chelate treatments occurred within the first 14 days of the experiment (Fig. 1). Maximum hatch in deionized water was 26.8%, whereas maximum hatch in reagent-grade zinc sulfate and zinc chloride was 64.3 and 46.5%, respectively. Hatching in both reagent-grade zinc solutions was higher ($P < 0.05$) than hatching in deionized water beginning on day 12; however, hatching in zinc chloride was lower ($P < 0.05$) than hatching in zinc sulfate. Egg hatch in the commercial zinc sulfate fertilizer treatment was 63.5% at the end of the experiment. Maximum egg hatch in zinc chelate was 17.7%, which was less ($P < 0.05$) than hatching in all other treatments including the deionized water control. In the second experiment, the zinc chelate treatment elicited the least amount of hatching, but was not different from the deionized water treatment.

Greenhouse experiments: Cumulative percentage egg hatch increased steadily for all treatments for 28 days (Fig. 2); little additional hatch was detected at day 60 (data

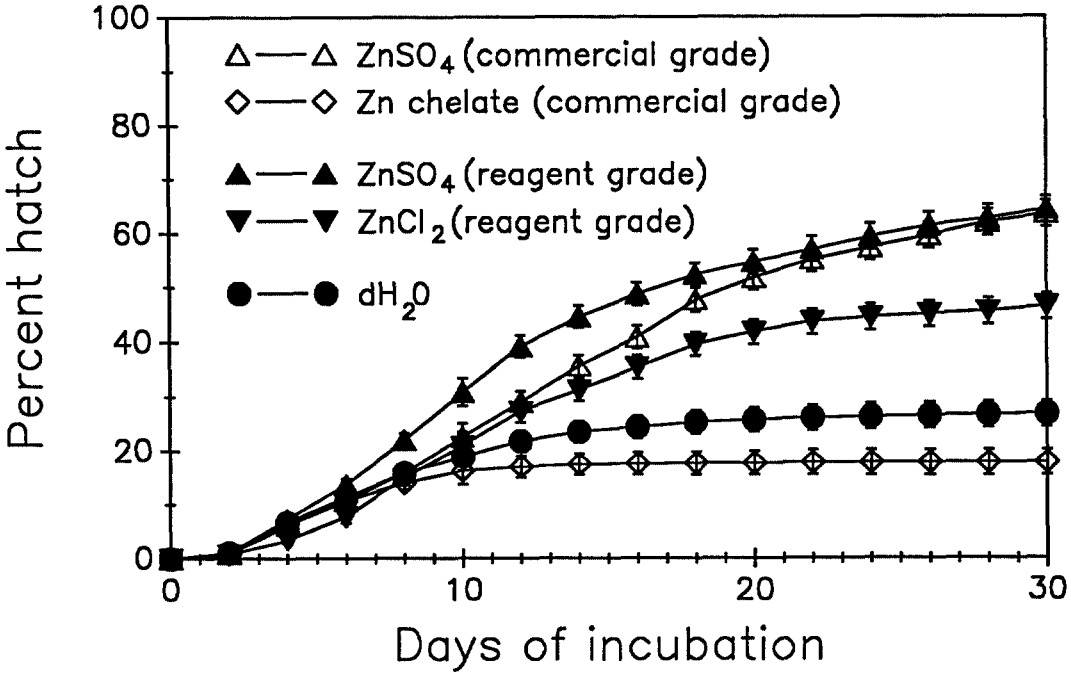

FIG. 1. Effects of 3 mM zinc solutions of reagent-grade zinc sulfate ($ZnSO_4$), zinc chloride ($ZnCl_2$), commercial-grade zinc sulfate (31 Plus Zinc Sulfate), zinc chelate (Sequestrene) fertilizer, and a deionized water (dH_2O) control on *Heterodera glycines* egg hatch for 30 days, in vitro. Error bars represent standard errors of the mean.

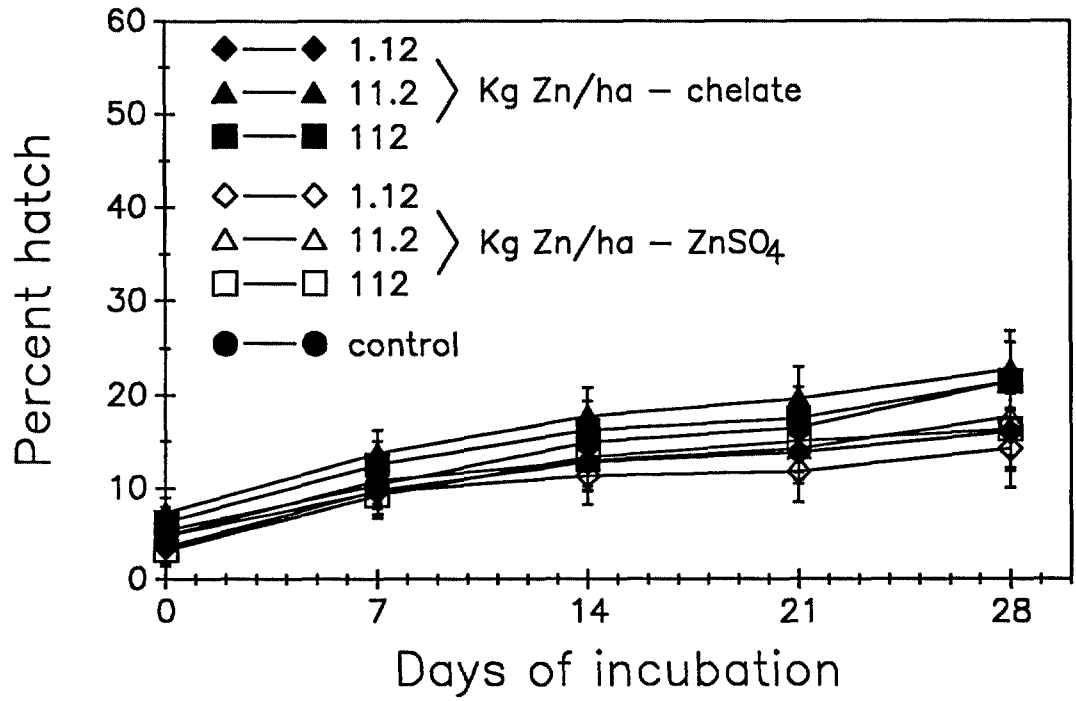

FIG. 2. Effects of three rates of soil-applied commercial zinc sulfate ($ZnSO_4$) and zinc chelate (Zn chelate) fertilizer on cumulative percentage hatch of *Heterodera glycines* eggs in a greenhouse experiment. Error bars represent standard errors of the mean.

not shown). Final cumulative percentage egg hatch ranged from 15.7% for the 1.12 kg Zn/ha zinc sulfate treatment to 27.2% for the 11.2 kg Zn/ha zinc chelate treatment. No differences ($P > 0.05$) in cumulative percentage egg hatch were detected at any individual sampling date. Average reproductive factors (Rf) ranged from 0.86 for the 1.12 kg Zn/ha zinc sulfate treatment to 0.55 for the 11.2 kg Zn/ha zinc chelate treatment (Fig. 3); however, there were no differences ($P > 0.05$) detected in Rf among any treatment combinations.

Overall average shoot dry weight for all treatments was 26.6 g. Average shoot dry weights for individual treatments ranged from 22.8 g for zinc chelate at 112 kg Zn/ha to 28.6 g for zinc sulfate at 1.12 kg Zn/ha (Fig. 4). There were no differences ($P > 0.05$) in shoot dry weight among treatments.

Initial DTPA-extractable zinc concentrations in soil averaged 12.1 kg Zn/ha (Table 1), with no differences ($P > 0.05$) among treatments. Beginning on day 7 and persisting throughout the experiment, concentrations of DTPA-extractable zinc were greater ($P < 0.0001$) in all zinc-fertilized treatments than in the unfertilized control treatment, and concentrations of extractable zinc were greater ($P < 0.0001$) in treatments fertilized with zinc sulfate than zinc chelate. Zinc concentrations remained relatively constant throughout the experiment.

Field experiments: Egg densities decreased during the growing season in all plots planted with corn in 1992 and increased in 1993 (Table 2). There were no consistent differences in *H. glycines* egg densities at planting and harvest detected among the treatments. The only difference in egg densities detected at any sampling date in both years was among mean egg densities at harvest in corn plots in 1993; egg den-

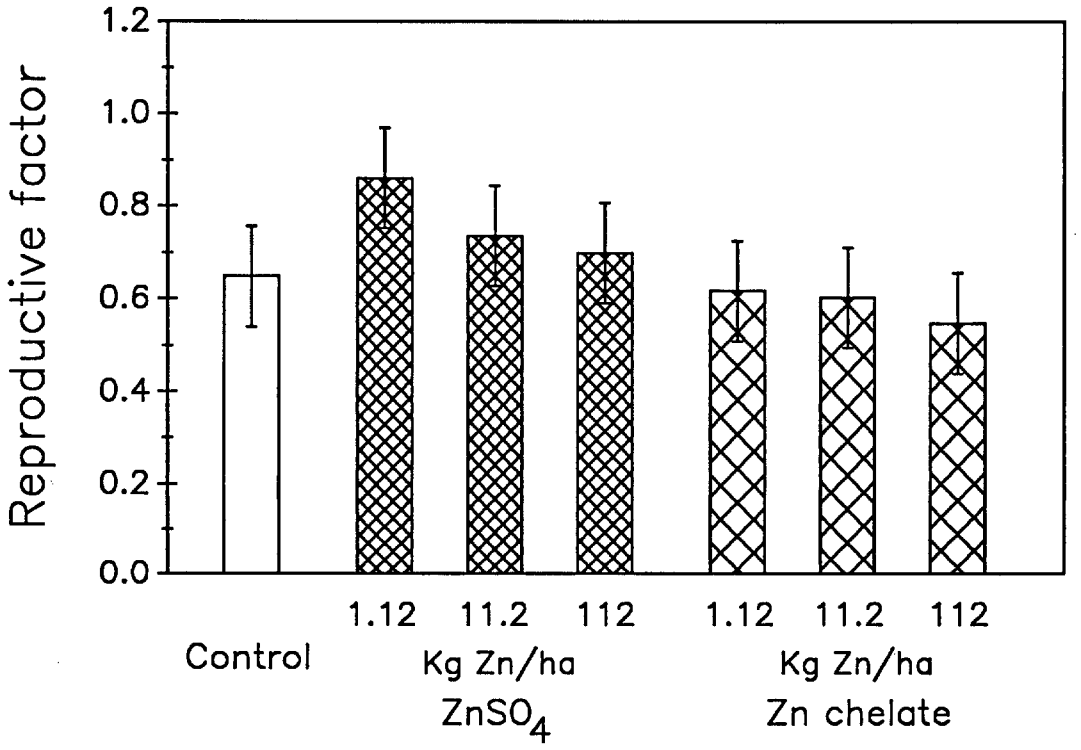

FIG. 3. Effects of three rates of soil-applied commercial zinc sulfate ($ZnSO_4$) and zinc chelate (Zn chelate) fertilizers on reproductive factors (final egg and J2 densities/initial egg and J2 densities) of *Heterodera glycines* after 60 days in the greenhouse. Error bars represent standard error of the mean.

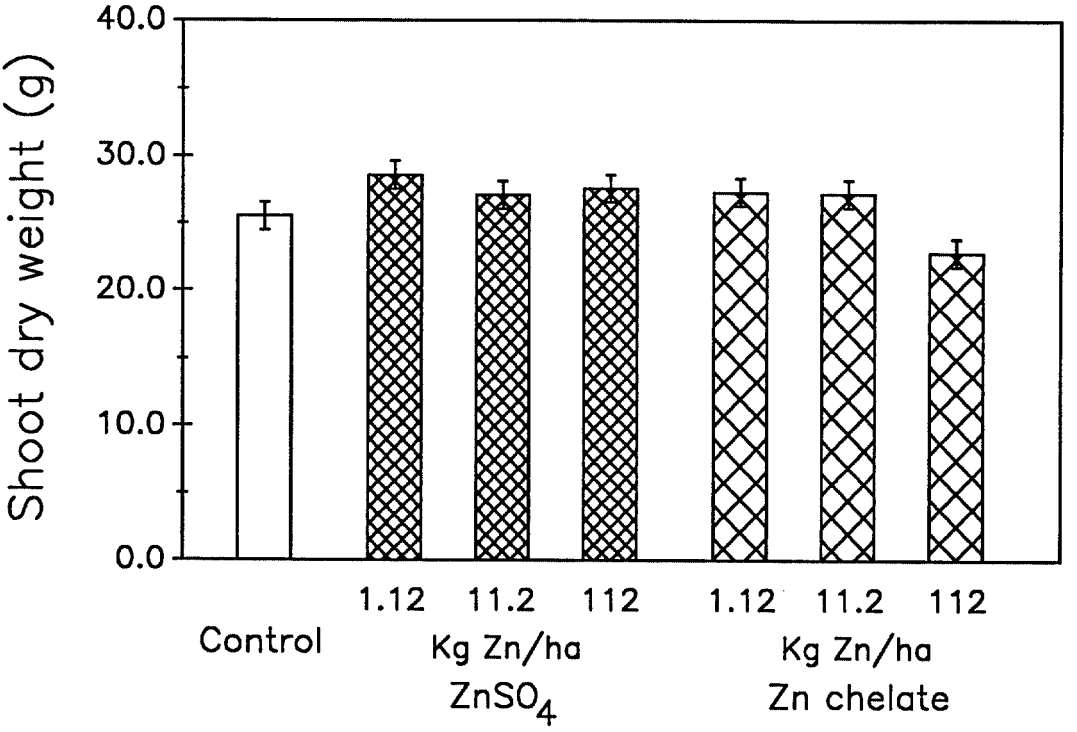

FIG. 4. Effects of three rates of soil-applied commercial zinc sulfate ($ZnSO_4$) and zinc chelate (Zn chelate) fertilizer on corn shoot dry weight at 60 days in the greenhouse. Error bars represent standard error of the mean.

sities in plots fertilized with zinc were greater ($P = 0.05$) than egg densities in unfertilized control plots (Table 2).

Corn and soybean yields were not affected by zinc fertilization. Corn yields ranged from 10,696 to 10,794 kg/ha in 1992 and from 7,474 to 8,085 kg/ha in 1993 (Table 3), but no differences ($P > 0.05$) among treatments were detected in either year. Likewise, mean soybean yields

TABLE 1. Diethylenetriaminepentaacetic acid (DTPA)-extractable zinc content of soil in greenhouse pots before and after application of zinc fertilizers.

Zinc source	Rate (Kg Zn/ha)	Kg extractable Zn/ha			
		Day 0†	Day 7	Day 28	Day 60
Control	0.00	15.0	15.7	18.1	11.6
Chelate	1.12	10.8	14.7	16.1	14.8
	11.20	13.2	20.1	23.2	17.3
	112.00	10.2	64.1	64.1	73.6
Mean		11.4	33.0	34.5	35.2
Sulfate	1.12	14.3	17.0	14.7	13.2
	11.20	10.3	22.5	22.8	19.5
	112.00	11.0	69.8	68.1	74.3
Mean		11.9	36.4	35.2	35.7
Contrasts ($P > F$):					
Control vs. others		0.38	0.0001	0.0001	0.0001
Sulfate vs. chelate		0.19	0.0001	0.0001	0.0001
Linear rate effect		0.88	0.0001	0.0001	0.0001

Data presented are means of five replications per treatment.
† Soil samples at Day 0 were collected before application of zinc fertilizers.

TABLE 2. *Heterodera glycines* egg population densities in plots planted with corn and soybean and fertilized with three levels of zinc chelate in 1992 and 1993.

Rate (Kg Zn/ha)	1992 Corn		1993 Soybean		1993 Corn	
	Pi†	Pf‡	Pi	Pf	Pi	Pf
0.0	30,128	24,394	3,674	10,454	3,638	3,727
11.2	17,819	11,211	5,843	5,955	4,878	7,946
22.4	13,003	9,400	4,490	7,013	4,536	5,750
$P > F$§	0.07	0.26	0.85	0.14	0.61	0.05

Data presented are means of five replications per treatment. Linear contrast computed over zinc application rate was not significant for any Pf.
† Pi = initial population (eggs per 100 cm^3 soil) at planting.
‡ Pf = final population (eggs per 100 cm^3 soil) at harvest.
§ Comparison of zinc-treated vs. untreated plots.

among treatments were not different and ranged from 3,440 to 3,569 kg/ha in 1993 (Table 3).

DISCUSSION

Commercial zinc sulfate fertilizer was as effective as reagent-grade zinc sulfate in stimulating *H. glycines* egg hatch in vitro. In our experiments, commercial zinc chelate fertilizer inhibited hatching. It is likely that the hatch inhibition was caused by the chelating agent, although our investigations did not examine the effects of the chelating agent alone. Tefft and Bone (13) also reported reduced *H. glycines* egg hatch in solutions containing zinc chloride plus various chelators relative to a water control treatment.

Stimulation of *H. glycines* hatching in soil in response to application of zinc fertilizers was not observed in our greenhouse and field experiments. No differences in *H. glycines* soil egg hatch or egg population densities were detected among treatments. Recommended rates of zinc applied as fertilizer to corn range from 1.12 to 22.4 kg Zn/ha (8,11); however, corn growth and yield were not stimulated in our greenhouse and field experiments.

Clarke and Shepherd (4) hypothesized that soil applications of zinc would not be practical for management of cyst nematodes because zinc cations are readily adsorbed to clay particles in the soil, and free zinc cations are necessary for stimulation of egg hatch. The extractable zinc content of the soil in our greenhouse experiments was increased significantly by direct application of zinc fertilizers, and repeated sampling and analysis of the soil for extractable zinc throughout the experiment indicated that no detectable zinc was lost from the pots. Although we were unable to determine the concentration of zinc cations in the soil, it is likely that the lack of hatch stimulation in these experiments was due to the unavailability of free zinc cations, as predicted by Clarke and Shepherd (4). We obtained similar results from greenhouse experiments conducted in a silty clay loam soil with a pH of 6.0 and from experiments in a field of Mexico silt loam with a pH of 7.0 in Missouri. It is possible that stimulation of *H. glycines* egg hatch in response to application of zinc fertilizers may occur in more acidic or coarse-textured soils, which typically have less cation exchange capacity, resulting in more free cations in the soil solution (14).

TABLE 3. Corn and soybean grain yield (kg/ha) from plots receiving three levels of fertilization with zinc chelate in 1992 and 1993.

Rate (Kg Zn/ha)	1992	1993	
	Corn	Soybean	Corn
0.0	10,720	3,548	8,085
11.2	10,794	3,569	7,474
22.4	10,696	3,440	7,608

Data presented are means of five replications per treatment. Means are not different ($P > 0.05$) according to single-degree-of-freedom comparisons.

In summary, stimulation of *H. glycines* hatching in solutions containing zinc ions is useful as a positive control treatment for laboratory hatch experiments. However, our experiments indicate that this phenomenon is not likely to be exploited for managing *H. glycines* in infested fields. Applications of zinc chelate and sulfate fertilizers to soil are ineffective at stimulating hatching of *H. glycines*.

Literature Cited

1. Acedo, J. R., and V. H. Dropkin. 1982. Technique for obtaining eggs and juveniles of *Heterodera glycines*. Journal of Nematology 14:418–420.

2. Alexander, R. D., K. A. McVay, and D. D. Buchholz. 1991. Zinc and sulfur on corn. Agronomy Miscellaneous Publication No. 91-02. Missouri Soil Fertility and Fertilizers Research Update 1990. University of Missouri, Columbia, MO.

3. Byrd, D. W., Jr., K. R. Barker, H. Ferris, C. J. Nusbaum, W. E. Griffin, R. H. Small, and C. A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. Journal of Nematology 8:206–212.

4. Clarke, A. J., and A. M. Shepherd. 1966. Inorganic ions and the hatching of *Heterodera* spp. Annals of Applied Biology 58:497–508.

5. Cochran, W. G., and G. M. Cox. 1957. Experimental designs, 2nd ed. New York: Wiley.

6. Doupnik, B., Jr. 1993. Soybean production and disease loss estimates for north central United States from 1989 to 1991. Plant Disease 77:1170–1171.

7. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

8. Killorn, R. J. 1984. Zinc—an essential nutrient. Iowa Cooperative Extension Service Pm-1129, Iowa State University, Ames, IA.

9. Niblack, T. L., ed. 1993. Protect your soybean profits: Manage soybean cyst nematode. Columbia, MO: University of Missouri Printing Services.

10. Niblack, T. L., R. D. Heinz, G. S. Smith, and P. A. Donald. 1993. Distribution, density, and diversity of *Heterodera glycines* in Missouri. Journal of Nematology 25:880–886.

11. Overdahl, C. J. 1978. Background for micronutrient needs. Pp. 25–28 *in* Soils, soil management and fertilizer monographs. Special Report 24. University of Minnesota, St. Paul, MN.

12. Sciumbato, G. L. 1993. Soybean disease loss estimates for the southern United States during 1988–1991. Plant Disease 77:954–956.

13. Tefft, P. M., and L. W. Bone. 1984. Zinc-mediated hatching of eggs of soybean cyst nematode, *Heterodera glycines*. Journal of Chemical Ecology 10: 361–372.

14. Tisdale, S. L., W. L. Nelson, and J. D. Beaton. 1985. Soil fertility and fertilizers, 4th ed. New York: Macmillan Publishing.

15. Tylka, G. L., T. L. Niblack, T. C. Walk, K. R. Harkins, L. Barnett, and N. K. Baker. 1993. Flow cytometric analysis and sorting of *Heterodera glycines* eggs. Journal of Nematology 25:596–602.

16. Vanden Heuvel, R. M., J. E. Sawyer, M. A. Schmitt, R. G. Hoeft, and G. S. Brinkman. 1989. Corn response to zinc on Illinois soils. Journal of Fertilizer Issues 6:68–76.

17. Winstead, N. N., C. B. Skotland, and J. N. Sasser. 1955. Soybean cyst nematode in North Carolina. Plant Disease Reporter 39:9–11.