DECOMPOSITION OF DEAD EGGS OF *HETERODERA GLYCINES* IN SOILS

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ABSTRACT

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A greenhouse experiment was conducted to study the rate of decomposition of autoclave-heating-killed eggs of the soybean cyst nematode, *Heterodera glycines*, in soil. The experiment was a factorial design including three soil pH levels and five treatment combinations of heating and amendments of liquid swine manure or untreated field soil that contained natural microbial communities. Soil pH affected the decomposition of *H. glycines* eggs contained in cysts, while no effect was detected for the amendments of swine manure and untreated field soil. The rate of decomposition was greater in the lower pH soil at 5.6 and 6.7 than in the pH 7.8 soil. In the pH 7.8 soil, it took approximately 450 d to reduce egg numbers below detectable levels, while in the pH 5.6 and 6.7 soils, the egg number declined to 0 at about 270 d after the experiment was set up. The predicted percentages of eggs remaining at 2 mon were 25% and 34% in the pH 5.6 and 6.7 soils and pH 7.8 soil, respectively. This study demonstrated that in some greenhouse studies of cyst nematodes, the residual dead eggs in soil may need to be measured for accurate determination of live egg population densities.

Key words: decomposition, *Heterodera glycines*, nematode eggs, soil pH, soybean cyst nematode, swine manure.

RESUMEN

Zhao, X., y S. Chen. 2015. Descomposición de huevos muertos de *Heterodera glycines* en suelo. Nematropica 45:113-117.

Se llevó a cabo un experimento en invernadero para estudiar la velocidad de descomposición en suelo de huevos de *Heterodera glycines* muertos por calentamiento en autoclave. El experimentó consistió en un diseño factorial incluyendo tres niveles de pH del suelo y cinco combinaciones de tratamientos térmicos y purines, además de suelo de campos no tratados que contenía comunidades microbianas naturales. El pH del suelo afectó a la descomposición de los huevos de *H. glycines* dentro de los quistes, sin embargo no se observó efecto alguno de los purines ni en el suelo no tratado. La velocidad de descomposición fue mayor a pHs del suelo bajos, (5.6 y 6.7) que al pH del suelo de 7.8. A pH 7.8, se necesitaron aproximadamente 450 d para reducir el número de huevos por debajo de los niveles de detección, mientras que a los pH del suelo 5.6 y 6.7, el número de huevos bajó hasta 0 en aproximadamente 270 d. Los porcentajes de huevos presentes en suelo estimados tras 2 meses fueron 25% y 34% a los pH del suelo 5.6 y 6.7, respectivamente. Este estudio demuestra que en algunos estudios en invernadero sobre nematodos quísticos, los huevos muertos residuales en suelo necesitan ser estimados para obtener una determinación precisa de las densidades de población de huevos vivos.

Palabras clave: descomposición, *Heterodera glycines*, huevos de nematodos, pH del suelo, nematodo quístico de la soja, purines.

It is common to use soils collected from crop fields for greenhouse experiments. These soils may be infested by plant-parasitic nematodes. Depending on the purpose of the greenhouse experiments, the soil may or may not be heat-treated to kill live nematodes and other organisms before use (Ferris, 1974; Chen *et al.*, 1995). In some experiments, field soils infested with nematodes are autoclave-heating-treated. Host plants growing in the soil may be inoculated with live nematodes, and after a period of time, the nematode population densities are measured (Xiao *et al.*, 2007). Heat-killed nematode cadavers

may remain in the soil for a period of time, and interfere with nematode population measurement in the experiments. The presence of residual autoclave-heating-killed nematodes in the soil is often ignored when measuring the nematode population density. This can be a concern especially for the study of egg population densities of cyst nematodes because the eggs in the cysts, either dead or alive, can be recovered resulting in inaccurate measurement of experimental live egg population densities. If a field soil contains a high egg population density, the number of residual dead eggs must be taken into consideration. The aim of the present study was to determine decomposition rate of heat-killed cyst nematode eggs in various pH soils under greenhouse conditions.

Three soil samples of Webster clay loam designated as Soil 1, Soil 2, and Soil 3 were collected from a field at three separate locations that differed in pH but had a similar texture: Soil 1 contained 5.3% of organic matter (OM), 38% sand, 32% silt, and 30% clay, with pH 5.6; Soil 2 contained 4.9% OM, 33% sand, 32% silt, and 35% clay, with pH 6.7; and Soil 3 contained 6.2% OM, 33% sand, 32% silt, and 35% clay, with pH 7.8. The soil was screened through a 5-mm-aperture sieve and mixed thoroughly. Initial egg population densities of the soybean cyst nematode, *Heterodera glycines*, were determined by cyst extraction and maceration. Soil 1, Soil 2, and Soil 3 contained 2,133, 1,333, and 8,833 eggs/100 cm^3 soil, respectively. The soil was treated with either autoclave-heating at 121°C for 1 hr to kill nematodes and other soil organisms, or without heating. After the autoclaving treatment, 1,933, 1,500, and 8,733 eggs/100 cm^3 soil were recovered from Soil 1, Soil 2, and Soil 3, respectively. A fresh liquid swine manure sample containing 4-5% of solid materials was collected from a finishing barn, equipped with a pull-plug manure handling system, at the University of Minnesota Southern Research and Outreach Center at Waseca, Minnesota, USA (Xiao *et al.*, 2007).

The experiment was a factorial design including three soil pH levels and five soil treatments with three replicates. The soil was placed in 20-cm-diam. clay pots (2.3 liters of soil/pot). The soil treatments were: 1) autoclaved field soil without amendment; 2) autoclaved field soil amended with liquid swine manure at 50 ml/liter of soil; 3) autoclaved field soil amended with liquid swine manure at 200 ml/liter of soil; 4) autoclaved field soil amended with 1% nonautoclaved field soil that contained no *H. glycines*; and 5) untreated field soil containing live *H. glycines* eggs.

The untreated field soil was mixed with the autoclaved soil thoroughly. The aim of the amendments of manure and untreated field soil was to determine whether the microbial organisms in the natural field soil and manure affect the rate of decomposition of the *H. glycines* dead eggs. The field soil without autoclaving treatment was used to compare the rate of decline of dead nematode eggs with that of live nematode eggs. During treatment, pots were half-filled with soil and amended with half the amount of manure or water. Additional soil was added to about 1 cm from the top of pot and the other half of manure or water was added on the soil surface.

The pots were maintained in the greenhouse with the temperature set at 28°C, and actual temperature recorded during September 2 to November 16, 2011, was 26.3°C ± 1.8°C (± SD). Water was added to keep soil moisture and other conditions similar to that used for other experiments (Xiao *et al.*, 2007; Liu and Chen, 2009). A sub-sample of approximately 100 cm^3 of soil was taken from each pot with a 2-cm-diam. soil sampling probe nine times (30- to 78- d intervals) within 441 d of the experiment (Table 1). Cysts were extracted from the soil, and egg densities in the soils were determined following the procedures used in the previous experiments (Xiao *et al.*, 2007; Liu and Chen, 2009).

In order to compare decline of the egg numbers in different soils, percentages of eggs were used instead of the actual egg densities (eggs/100 cm^3 soil). The percentages were calculated by dividing the egg density at a sampling date by the egg density at day 1. The data of each sampling time were examined for normality with Shapiro-Wilk plotting and test, and transformed to ln(x) to improve homogeneity of variance before being subjected to analysis of variance (ANOVA) using Statistix 8 (Analytica Software, Tallahassee, FL). Means were separated by the Tukey's Honestly Significant Difference (HSD) test at α = 0.05. Since there was no significant treatment effect of the soil amendments on the number of dead nematodes in the soil, the data of the four soil treatments were combined and multiple regression was performed to determine the relationship between percentage of dead nematode eggs in the soil with sampling time and soil pH.

There was no significant interaction between the soil pH and soil treatment except for the sampling time at 182 d after the pot setup, indicating the two factors were generally independent (Table 1). Although significant soil treatment effect was observed at 90 to 441 d after the experiment was set up, the differences were observed only between the live and dead nematode eggs; there was no difference between any soil treatments for the dead nematode eggs. The pH 7.8 soil generally had a higher percentage of nematode eggs recovered than the soils with lower pH of 5.6 and 6.7, especially in the later sampling dates (Table 1).

At 182 d, significant differences among the

Table 1. Percentages of *Heterodera glycines* eggs recovered over time from three different pH soils with various treatments of heating, swine manure amendments, and untreated field soil.

Treatments	Sampling time (days after pot setup)								
	31	62	92	123	182	237	308	363	441
Soil pH									
5.6	22.9 b[y]	11.7 b	11.2 ab	6.1 b	4.9 b	4.2 b	2.9 b	1.1 b	3.8 b
6.7	52.5 ab	42.0 a	16.2 b	11.8 b	15.8 b	12.7 b	5.6 b	3.1 b	6.6 b
7.8	61.0 a	52.6 a	26.7 a	26.0 a	27.5 a	23.3 a	22.0 a	17.9 a	16.08 a
Soil treatment [x]									
1	41.5 a	24.5 a	6.8 b	8.1 b	8.9 b	5.2 b	2.6 b	1.8 b	1.7 b
2	30.8 a	22.5 a	13.2 b	10.6 ab	7.0 b	3.9 b	2.6 b	3.0 b	1.2 b
3	42.4 a	26.5 a	13.6 ab	9.2 b	6.2 b	4.9 b	2.7 b	1.7 b	2.1 b
4	45.9 a	28.9 a	9.5 b	8.0 b	11.8 b	4.4 b	6.6 b	3.5 b	2.8 b
5	66.6 a	74.7 a	47.0 a	37.3 a	46.6 a	48.7 a	36.4 a	26.9 a	37.5 a
Analysis of Variance (*F*-values)[z]									
Soil (S)	4.8*	18.0****	4.1*	15.2****	16.2****	9.9***	35.7****	45.11****	13.22***
Soil treatment (T)	0.5	1.9	6.2**	6.2**	5.38**	8.8***	21.5****	20.86***	16.62****
S × T	0.5	0.6	1.8	1.4	2.6*	0.7	0.2	1.3	1.0

[x] Soil treatments: 1) autoclaved field soil without manure amendment; 2) autoclaved field soil amended with liquid swine manure at 50 ml/liter of soil; 3) autoclaved field soil amended with liquid swine manure at 200 ml/liter of soil; 4) autoclaved field soil amended with 1% nonautoclaved field soil that contained no *H. glycines*; and 5) untreated field soil containing live *H. glycines* eggs.

[y] The values followed by the same letter in a column are not significant according to Tukey's HSD test at α = 0.05.

[z] *, **, ***, and **** represent significant levels at $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

Table 2. Interactive effect of soil pH and treatments of heating, swine manure amendments, and untreated field soil on the percentage of *Heterodera glycines* eggs recovered 182 days after pot setup.

Soil treatment[y]	Soil pH		
	5.6	6.7	7.8
1	2.6 aB[z]	12.8 abAB	11.4 cA
2	4.5 aA	6.9 abA	9.35 cA
3	3.2 aAB	0.8 bB	14.4 bcA
4	7.1 aA	5.8 abA	22.5 bA
5	7.2 aA	52.8 aA	79.7 aA

[y] Soil treatments: 1) autoclaved field soil without manure amendment; 2) autoclaved field soil amended with liquid swine manure at 50 ml/liter of soil; 3) autoclaved field soil amended with liquid swine manure at 200 ml/liter of soil; 4) autoclaved field soil amended with 1% nonautoclaved field soil that contained no *H. glycines*; and 5) untreated field soil containing live *H. glycines* eggs.

[z] The values followed by the same lowercase letter in a column or the same uppercase letter in a row are not significant according to Tukey's HSD test at α = 0.05.
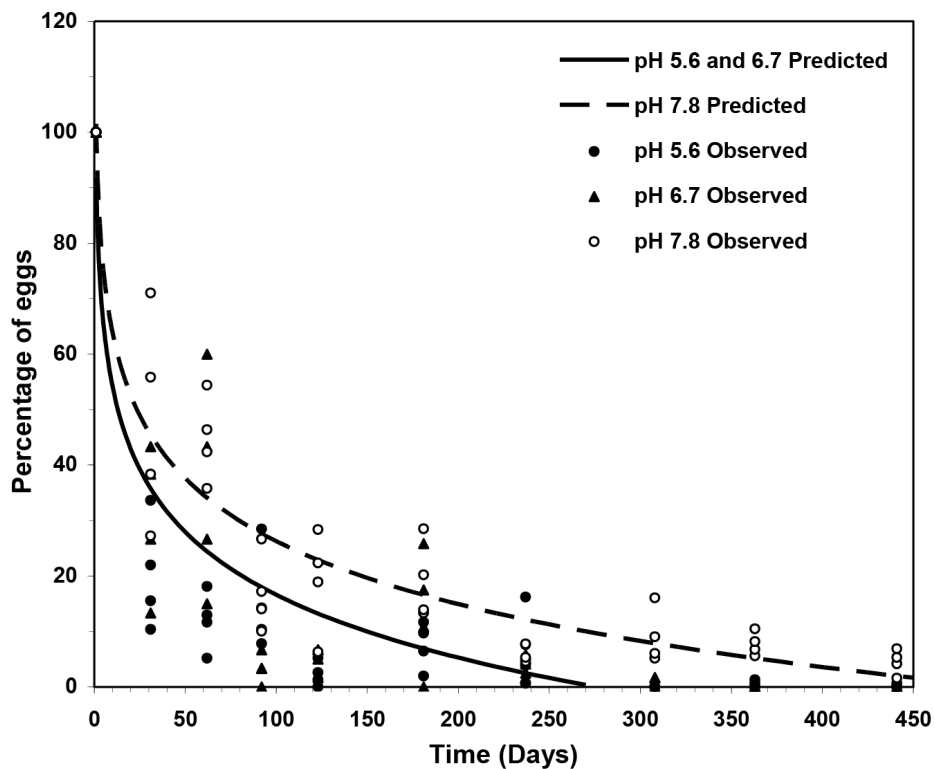
Fig. 1. Percentage of autoclave-heating-killed eggs of *Heterodera glycines* recovered over time from three soils having different pH levels. The percentage of eggs can be predicted with the model $Y = 91.887 − 16.346\ln x_1 + 9.647x_2$, where Y is the percentage of eggs as compared with initial egg population density in the soil, x_1 is time (days after the experiment was set up), and x_2 is soil pH (0 is pH 5.6 or 6.7, and 1 is pH 7.8), ($R^2 = 0.89$, $P < 0.0001$).
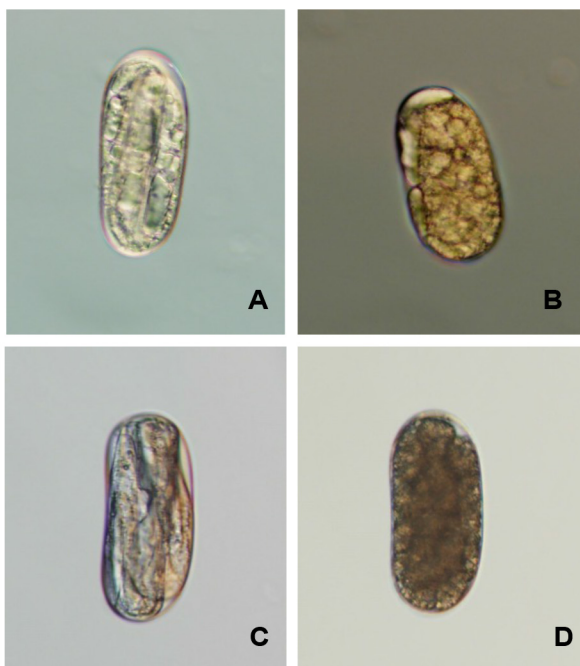

Fig. 2. Autoclave-heating-killed eggs recovered from potted field soil in the greenhouse after 3 months (A, B) and the live eggs from a yellow female from soybean root in a greenhouse pot culture (C, D).

soil treatments were observed in soil with pH 6.7 and 7.8, but not in pH 5.6 (Table 2). At pH 6.7, only the soil treatment 3 with 200 ml/liter manure resulted in lower egg number than the soil treatment 5 containing live eggs. At pH 7.8, the soil treatment 5 had higher number of eggs recovered than all other four soil treatments (1-4) containing dead eggs, and the soil treatment 4 with 1% non-autoclaved field soil had higher egg numbers than the soil amended with 50 ml/liter manure and without amendment. Differences in soil pH were observed only in soil treatment 1 without amendment and in treatment 3 with 200 ml/liter manure; the pH 7.8 soil had higher egg counts than the pH 5.6 in treatment 1 and pH 6.7 in treatment 3 (Table 2).

Multiple regression analysis revealed that the decomposition rate of the dead nematode eggs differed in the three soils of different pH (Fig. 1). There was no interaction between the soil pH and sampling time. However, the rate of decomposition was greater in the lower pH soils than the pH 7.8 soil. At the higher pH, multiple regression showed that it would take approximately 450 d to reduce egg numbers below detectable levels, while at soil pH of 5.6 and 6.7, the egg number declined to 0 at about 270 d. The predicted percentages of eggs remaining at 1 mon were 36% and 45%, and at 2 mon were 25% and 34% in the pH 5.6 and 6.7 soils and pH 7.8 soil, respectively. The shape and structure of the eggs remained similar to the eggs from live females, and it is difficult to distinguish them (Fig. 2).

In our literature search, we found no information on the decomposition rate of dead nematodes in either the greenhouse or natural field soils. In this study, a high number of cyst nematode eggs remained detectable in the soil in the greenhouse pots after one or two months even after the eggs were killed by the autoclave-heating. Decomposition of eggs in cysts may be affected by many factors. In the greenhouse soils, addition of manure or untreated field soil did not affect the decomposition rate of dead eggs but the soil pH affected the decomposition rate. The more acidic soils at pH 5.6-6.7 exhibited a higher egg decomposition rate than the more alkaline soil at pH 7.8. The reason for this is unclear. It is possible that the microbial activities under acidic conditions at the pH 5.6 and 6.7 were greater than under the alkaline condition at pH 7.8. However, in natural soil, bacterial population densities were greater in high pH soil than in the low pH soil and there was limited effect of pH on fungal population (Rousk *et al.*, 2010). Further studies are needed to determine why pH affected the decomposition of nematode eggs.

Although decomposition rate of nematodes under field conditions may be different from that in the greenhouse, this study may also be useful for measuring field nematode population densities. For example, in field evaluation of nematicide for control of cyst nematodes, the nematode egg population densities at 1 or 2 mon are often measured to determine the treatment effect. This could be confounded by the residual dead eggs that are killed by the nematicide. If the initial egg population density is high, dead eggs even after 1 or 2 months can mask the treatment effect. In this situation, alternative population measurements such as live second-stage juveniles in the soil or nematodes in plant roots may be more accurate and informative.

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