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Effect of Liquid Swine Manure on Hatch and Viability of *Heterodera glycines*

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Abstract: Experiments were conducted in the laboratory and greenhouse to determine the effect of raw and anaerobically digested liquid swine manures on the hatch and viability of *Heterodera glycines*, the soybean cyst nematode. Anaerobic digestion was performed for 15 and 35 days to enrich volatile fatty acids (VFA) and ammonium (NH_4^+), respectively. All filtrates of the raw, VFA-enriched, and NH_4^+-enriched manures at 10^{-1} to 250^{-1} dilutions inhibited *H. glycines* hatch, and the reduction of hatch was increased with increasing concentration of the manure. Cumulative hatch at day 21 was only 2.1% to 3.7% in the 10^{-1} dilution manures, while the hatch in water was 21% to 27.3%. The high concentrations appeared to be lethal to some eggs. Most second-stage juveniles (J2) of *H. glycines* were killed when incubated for 8 hours in the manure filtrate at the original concentration (>90% mortality) or for 48 hours at the 64^{-1} dilution (> 82% mortality). When J2 were treated with the manures at 10^{-1} to 250^{-1} dilutions for 4 hours, only the 10^{-1} dilution of VFA-enriched and raw manures resulted in a lower number of J2 that penetrated soybean roots as compared with lower concentrations. The VFA-enriched manure was the best, raw manure intermediate, and NH_4^+-enriched manure the least effective in inhibiting *H. glycines* hatch and killing eggs and J2.

Key words: ammonia, fatty acid, *Glycine max*, hatch, *Heterodera glycines*, mortality, swine manure, soybean, soybean cyst nematode.

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is one of the most important plant-parasitic nematodes. The soybean yield losses caused by *H. glycines* in the US were greater than those caused by any other disease (Wrather and Koenning, 2006). Although *H. glycines*-resistant soybean cultivars are widely used to manage this nematode, breeding and deployment of resistant cultivars is challenging due to the genetic variability of *H. glycines* (Niblack and Riggs, 2004), the selection pressure on *H. glycines* when resistance genes are used (Young et al., 1986; Young and Hartwig, 1992; Young, 1994) and the linkage of yield-suppressive factors with *H. glycines*-resistance genes (Mudge et al., 1996; Kopisch-Obuch et al., 2005). Although some nematicides are effective in lowering *H. glycines* population densities, they are costly and can have negative impact on the environment and human health (Thomason, 1987). Clearly, an integrated approach with multiple strategies is needed for the long-term effective management of *H. glycines*.

The use of agricultural waste plant and animal materials to achieve lowering nematode population densities and increasing tolerance of plants to nematodes is one potential alternative method for managing plant-parasitic nematodes (Rodriguez-Kabana et al., 1987; Barker and Koenning, 1998; Akhtar and Malik, 2000). A number of waste products such as oilcake, chitin, animal manures, green manures, yard wastes, municipal wastes and fishery wastes have been evaluated for their potential to control plant-parasitic nematodes, including *H. glycines*. For example, soil amendment with chitin may encourage the development of nematode-antagonistic soil fungi and bacteria, leading to suppression of *H. glycines* population densities (Rodriguez-Kabana et al., 1984; Tian et al., 2000). The addition of powdered pine bark to soil increased soil fungal population densities and suppressed *H. glycines* population densities (Kokalis-Burelle and Rodriguez-Kabana, 1994). Residues of certain plant species have been found to affect lipid utilization and infectivity of *H. glycines* J2 (Riga et al., 2001). Warnke et al. (2006, 2008) tested 46 plant species as rotation crops and green manures in the greenhouse for suppression of *H. glycines*, and they found that some crops effectively lowered *H. glycines* population density due to stimulation of J2 hatch and possible nematicidal effects on J2 and eggs. Recently, an alkaline-stabilized biosolid (N-Viro Soil) developed from municipal waste materials has been evaluated for managing *Meloidogyne* spp. and *H. glycines* in greenhouse and field studies (Koenning, 2004; Melakeberhan and Noel, 2004; Zasada and Tenuta, 2004; Zasada, 2005; Mennan et al., 2007; Zasada et al., 2008). The results were somewhat inconsistent; N-Viro reduced *H. glycines* population densities at high application rates in some studies, but there was no reduction of *H. glycines* population densities at low application rates. It appeared that N-Viro Soil released ammonia (NH_3) at high soil pH to kill nematodes (Zasada and Tenuta, 2004).

Another type of waste material tested against *H. glycines* is swine manure, which is widely available and has been used as fertilizer to improve plant growth and yield. Reynolds et al. (1999) reported that application of swine manure in fields improved soybean yield and resulted in higher *H. glycines* end-season population densities. In a laboratory study, some volatile compounds that can be found in swine manure inhibited *H. glycines* hatch and J2 movement (Reynolds et al., 1999). In yet another experiment, Stein et al. (2000) found that ozonated swine manure was more effective in in-

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hibiting *H. glycines* hatch and killing J2 than unozonated swine manure.

Fresh swine manure normally contains a large amount of proteins that can be converted via anaerobic digestion to volatile fatty acids (VFA) and ammonium (NH_4^+) (Ndegwa et al., 2002). Volatile fatty acids and ammonia (NH_3), which can be transformed from ammonium in the manure, have nematicidal activities (Rodriguez-Kabana, 1986; Gommers and Bakker, 1988; Chitwood, 2002). The concentration of VFA and NH_4^+ may depend on the source of swine manure and length of digestion (Conn et al., 2007; Xiao et al., 2007a). It has been reported that the concentration of VFA in liquid animal manure changes during anaerobic digestion (Spoelstra, 1979; Guenzi and Beard, 1981; Rainville and Morin, 1985; Ndegwa et al., 2002).

In order to obtain high concentrations of VFA and NH_4^+ (for conversion to NH_3) for maximum *H. glycines* suppression, we conducted an experiment to determine the concentrations of these compounds during anaerobic digestion (Xiao et al., 2007a, 2007b). The length of anaerobic digestion necessary for the liquid swine manure to reach the highest levels of VFA and NH_4^+ were 17 to 18 and 28 to 46 days, respectively. In a subsequent experiment, VFA-enriched and NH_4^+-enriched liquid swine manures collected at 15 and 35 days of anaerobic digestion, along with the raw manure without the anaerobic digestion treatment, were evaluated for their effectiveness in suppressing *H. glycines* population densities in the greenhouse. The study showed that the VFA-enriched manure was the best, raw manure the second, and the NH_4^+-enriched manure the least effective in lowering *H. glycines* population densities (Xiao et al., 2007b). The mechanisms involved in suppression of nematode population densities by the different kinds of swine manures remain unclear. The objective of this study was to determine the effects of VFA-enriched, NH_4^+-enriched and raw swine manures on the hatch, viability and infectivity of *H. glycines* J2.

Materials and Methods

Manure preparation: Raw, VFA-enriched and NH_4^+–enriched liquid swine manures were prepared following procedures described previously (Xiao et al., 2007a). Briefly, fresh manure was collected directly with a continuous collection system for 2 wk in a finishing barn located at the Southern Research and Outreach Center in Waseca, MN, without going through the anaerobic digestion process. The fresh manure was then anaerobically digested in a 4.5-m^3 tank, and samples were taken from the tank at 15 and 35 d as VFA- and NH_4^+–enriched manures, respectively. Fresh manure without going through the digestion process was used as raw manure. All collected materials were stored in a freezer at −20°C if not used immediately after collection. The concentrations of VFA, NH_4^+ and total solids, and pH of the manures were measured following procedures described previously (Xiao et al., 2007a) and are summarized in Table 1.

Manure filtrate preparation: For filtrate preparation, solid materials were initially removed from the liquid manure by centrifugation for 15 min at 1,500*g*, and then the solutions were passed through 2.7-µm- and 1.2-µm-pore filter papers, followed by 0.45-µm- and 0.2-µm-pore syringe-tip filters to further remove remaining microbial spores and cells before storage at 4°C until use (Warnke et al., 2008).

Hatch assay: The effect of the manures on hatch of the *H. glycines* was quantified following procedures reported previously (Chen et al., 2000; Warnke et al., 2008). *Heterodera glycines* (race 3, HG Type 0-) was cultured on susceptible soybean 'Sturdy' for one and a half months. Newly formed females and cysts were washed from roots with a vigorously applied water stream through an 850-µm-aperture sieve to remove root debris and collected on a 250-µm-aperture sieve. Cysts were then extracted from soil and root debris by centrifugation in 63% (w/v) sucrose solution at 1,500*g* for 5 min. Eggs were released from the cysts by crushing the cysts on a 150-µm-aperture sieve with a rubber stopper mounted on a motor (Faghihi and Ferris, 2000). The eggs were separated from debris by centrifugation in a 10% and 38% (w/v) sucrose solution for 5 min at 1,500*g* to remove most of the empty eggs and remaining debris. The collected eggs were transferred onto a 38-µm-pore sieve, rinsed with sterile water and then treated with the antibiotic SCQ solution (100 ppm streptomycin sulfate, 50 ppm chlortetracycline, and 20 ppm 8-quinolinol) for 24 hr at 4°C. A suspension of 2,200 eggs/ml was prepared in sterile water after the eggs were rinsed with sterile water. One milliliter of egg suspension including 2,200 eggs was placed on a 1-cm-diam., 35-µm-pore sieve. The sieves with eggs were placed in a well of 24-well tissue culture plates, and only the center well of each plate was used. The raw, VFA-enriched and NH_4^+–enriched manures were diluted to 10^{-1}, 50^{-1} and 250^{-1} with sterilized deionized water and added to the tissue culture plates in a quantity (about 2 ml) that just reached the bottoms of the sieves. Sterilized deionized water and 4 mM $ZnCl_2$ solutions (a hatch stimulant) (Tefft and Bone, 1984) were included as controls. Each plate was placed in a container separately in order to prevent the volatile compounds of the

Table 1. Concentrations of VFA, NH_4^+ and total solids, and pH of the manures used in the experiments.

Manure	pH	VFA (mg/liter)	NH_4^+ (mg/liter)	Total solid (g/liter)
Raw	7.38	6,040	1,520	49.1
VFA-enriched	7.40	6,740	1,720	20.7
NH_4^+-enriched	7.58	4,560	1,840	18.7

manure samples from affecting each other, and there was a layer of water in the covered container to minimize evaporation and loss of the solution. The containers were maintained at room temperature (approx. 22–24°C). Three replicates were used for each treatment. At d 3, 7 and 14, the hatch solutions were replaced with the stored fresh solution, and the J2 that hatched in the solutions were counted. At d 21, all solutions were replaced with 4 mM $ZnCl_2$, and the J2 that hatched were counted. The $ZnCl_2$ solution was replaced with a fresh one every 7 d until most (>70%) eggs in the no-manure treatment had hatched. The extended period of incubation of the eggs in $ZnCl_2$ served to determine the viability of eggs after 21 d of manure exposure (Chen et al., 2000; Warnke et al., 2008). The experiment was performed twice.

J2 viability assay: Eggs were prepared following the procedures described above. For hatching, the eggs were placed on 35-µm-aperture filter cloth shallowly submerged in 4 mM $ZnCl_2$ and incubated at 24°C. Juveniles that emerged within each of the 2 d (after discarding J2 that hatched in the first 3 d) were collected on a 15-µm-aperture sieve. In order to obtain the most active J2, three layers of coffee filter papers were placed on a screen in a dish. The collected J2 were poured onto the filter papers, and enough water was added to the dish so that the filter papers were shallowly submerged. Those J2 that moved down through the filter papers within about 3 hr were likely the most active individuals and were used for viability assays (Warnke et al., 2008). Fifty J2 in 0.1 ml deionized water were placed in a well of a 24-well tissue culture plate, and 1.5 ml of the treatment solution, either raw, VFA-enriched or NH_4^+-enriched manure at 1, 4^{-1}, 16^{-1} or 64^{-1} dilution, or deionized water control, was added into the wells. A randomized block design with three replicates was used. The J2 in the manures were incubated at 28°C for 8, 24 and 48 hr. After the incubation periods, dead and alive J2 were observed and quantified using an inverted microscope. The viability of J2 was determined by adding a drop of 1N NaOH. The J2 that changed shape from straight to curled or hook-shaped within 1 min were considered viable (Chen and Dickson, 2000). There were two experimental runs.

J2 infectivity assay: The procedures for preparation of J2 were the same as in the viability assay. The raw, VFA-enriched and NH_4^+–enriched manures were diluted to 10^{-1}, 50^{-1} and 250^{-1}, and deionized water was included as a control. Aliquots of J2 (3,000 in 0.3 ml deionized water) were placed in 50-ml centrifuge tubes, and 3.7 ml manure solutions or water were added. The J2 in the 4 ml of water or manure solutions were incubated at room temperatures (22–24°C) for 4 hr before being inoculated along with manure solution on 1-wk-old soybean seedlings in a 100-cm^3 cone-tainer (Stuewe & Sons, Inc., Corvallis, OR) which contained 100 cm^3 of the mixture of 80% clay loamy soil and 20% fine sand. The cone-tainers were maintained in the greenhouse at 25°C and watered after 12 hr and once every 12 hr thereafter to maintain adequate soil moisture. After 48 hr, soybean roots were removed, washed gently, placed in a 50-ml centrifuge tube containing water and frozen at −20°C for 24 hr. The roots were thawed and then blended in water for 30 sec to release J2. The J2 were separated from most debris by passing through a 250-µm-aperture sieve onto a 38-µm-aperture sieve and then by centrifugation in a 38% (w/v) sucrose solution at 1,500*g* for 5 min. The J2 were stained with acid fuchsin (Byrd et al., 1983) and counted using an inverted microscope. Five replicates were used, and the experiment was performed twice.

Statistical analysis: For the hatch assay, the cumulative percentage hatch was calculated for the individual sampling times. The percentage hatch data were transformed with ln-degree-asin ($x^{0.5}$) to improve homogeneity of variance before being subjected to analysis of variance (ANOVA) using SAS 9.1 linear procedure (SAS Institute Inc., Cary, NC). For the J2 viability study, the calibrated mortality of J2 caused by manure treatment was determined: Mortality = 100 × (% alive J2 in water control – % alive J2 in manure treatment)/% alive J2 in water control. The percentage mortality was transformed with degree-asin ($x^{0.5}$) prior to ANOVA. The numbers of J2 penetrated roots were transformed with ln (x) prior to analysis. Initially, the data of the two repeated runs were pooled for analyses of any interaction between the run and manure treatments. Because significant interactions were observed for experiments in hatch and J2 mortality assays, the data of the two runs of the two assays were further analyzed separately with the linear model procedure. Significant mean separation was determined with Fisher's least significant difference (LSD) test at $\alpha = 0.05$. In addition, regressions were performed to determine the relationship between the percentage reduction of egg hatch at d 21 and the dilution of manure and to determine the interactive effect of manure type and dilution on the hatch reduction. The percentage reduction of hatch equals 100 × (nematodes hatched in water – nematodes hatched in manure treatment)/nematodes hatched in water. The model used for the regression was: $\ln Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_1 \cdot X_2 + \beta_6 X_1 \cdot X_3 + \beta_7 X_1 \cdot X_4 + \beta_8 X_2 \cdot X_4 + \beta_9 X_3 \cdot X_4 + \beta_{10} X_1 \cdot X_2 \cdot X_4 + \beta_{11} X_1 \cdot X_3 \cdot X_4$, where Y = percentage reduction of hatch as compared with water control, $X_1 = \ln$(dilution of manure), $X_2 = 1$ if raw manure and 0 if others, $X_3 = 1$ if VFA-enriched manure and 0 if others, $X_4 = 1$ if Run 1 and 2 if Run 2, β_0 is the constant, and β_1 to β_{11} are the coefficients of corresponding terms.

Results

Effects on hatch: General trends of the data in the two runs were similar, although the interactive effect of the

manure treatment and run was significant. Overall, all manures at the three dilutions inhibited hatch compared to the water control at all sampling dates within 21 d (Table 2). At d 3, only 1.5% to 5.8% of J2 hatched from eggs in different manure treatments; in contrast, 7.1% (Run 2) to 12.1% (Run 1) of the J2 hatched in water. Cumulative hatch at d 21 was only 3.7%, 2.6% and 2.8% in Run 1 and 3.2%, 2.1% and 3.4% in Run 2 for the 10^{-1} dilution of raw, VFA-enriched and NH_4^+-enriched manures, respectively, while the hatch in water was 27.3% in Run 1 and 21% in Run 2. The hatch rate was much higher in $ZnCl_2$ than in water, indicating that a large percentage of J2 in the eggs were dormant and the $ZnCl_2$ broke the dormancy. After all hatch solutions were replaced by $ZnCl_2$ at d 21, egg hatch increased greatly, especially in the low manure concentrations. At d 42, hatch in the water treatment (first 21 d) reached 62.6% in Run 1 and 72.5% in Run 2, similar to that in $ZnCl_2$. At this point in time, hatch in all manure treatments (first 21 d) except for the 250^{-1} dilution of NH_4^+-enriched manure, especially in the high concentrations, was still lower than that in water, suggesting that some eggs were probably killed by the manures; the hatch in treatment of 250^{-1} dilution of NH_4^+-enriched manure was 57.9% (in Run 1) and 57.6% (in Run 2), similar to that in water, suggesting that there was little mortality of eggs at the low concentration of NH_4^+-enriched manure.

The relationship between the reduction of cumulative hatch at d 21 and concentration of the three types of manure is expressed by the model: $\mathrm{Ln}Y = 5.108 + 0.265X_1 - 0.242X_2 - 0.204X_3 - 0.031X_4 - 0.088X_1 \cdot X_2 - 0.097X_1 \cdot X_3$ ($R^2 = 0.977$, $P < 0.0001$) (Fig. 1). Overall, the reduction of the hatch in the manure treatment as compared with water increased with increasing manure concentration. The VFA-enriched manure was more effective in lowering the hatch than the other two types of manure at all concentrations. NH_4^+-enriched manure had higher hatch reduction than the raw manure at high concentrations (e.g., 10^{-1} dilution), but had lower hatch reduction at low concentrations (e.g., 250^{-1} dilution) (Fig. 1).

Effects on J2 viability: Interaction between the manure treatment and run was significant, and the analyses of variances of the two runs are separately presented in Table 3. In Run 1, the main effects and interaction of the dilution and type of manure on the percent mortality of J2 were significant at incubation time of 8 and 24 hr. At 48 hr, the differences in J2 mortality were observed only among different dilution treatments. In Run 2, the differences in J2 mortality were significant among the dilutions at all three sampling points and among the manure types at the incubation time of 24 and 48 hr. In addition, interaction between type and dilution of manures was significant at 24 hr (Table 3).

Table 4 shows detailed interactive effects on J2 mortality and differences among the treatments. The treatment effect was more differentiated among the dilu-

TABLE 2. Cumulative percentage hatch of *Heterodera glycines* in solutions of manure filtrates. After 21 days, all eggs were incubated in $ZnCl_2$, a hatch stimulant. Inhibition of hatch by the manure filtrates is determined by comparing the cumulative hatch in the manure treatments with the hatch in water at day 3 to day 21. The egg viability is estimated by comparing the cumulative hatch at day 28 to day 42.

Manure	Dilution (x^{-1})	Day 3[a]	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
				Run 1				
Raw	10	2.0 f	2.8 g	3.2 h	3.7 h	8.1 g	9.6 e	10.6 f
	50	4.4 de	8.5 e	9.6 f	10.1 f	40.3 e	45.8 c	47.4 cd
	250	5.3 c	10.2 d	12.5 d	12.8 d	42.4 de	47.0 c	48.0 cd
VFA-enriched	10	1.5 g	2.1 h	2.4 i	2.6 i	5.8 h	7.1 f	7.8 g
	50	3.9 e	7.2 f	8.2 g	8.5 g	34.5 f	37.8 d	38.8 e
	250	5.2 cd	9.7 d	11.4 de	12.0 de	40.9 de	45.0 c	45.9 d
NH_4^+-enriched	10	1.5 g	2.4 h	2.6 i	2.8 i	8.2 g	10.0 e	11.0 f
	50	5.2 cd	9.1 de	10.3 ef	11.2 e	45.8 cd	50.2 c	51.7 c
	250	5.8 c	12.0 c	15.0 c	16.3 c	50.2 c	56.8 b	57.9 b
Water		12.1 b	19.7 b	25.4 b	27.3 b	57.4 b	61.4 b	62.6 b
$ZnCl_2$		22.1 a	44.4 a	60.6 a	66.9 a	68.5 a	69.6 a	70.1 a
				Run 2				
Raw	10	1.7 e	2.5 f	2.9 f	3.2 g	9.0 f	10.9 f	11.5 f
	50	3.9 d	6.6 de	7.5 e	7.9 f	42.3 cd	48.7 c	49.9 c
	250	5.6 c	8.7 c	9.7 cd	10.3 d	41.5 d	46.8 cd	47.9 cd
VFA-enriched	10	1.5 f	1.9 g	2.0 g	2.1 h	5.3 g	6.6 g	7.4 g
	50	3.4 d	6.0 e	7.0 e	7.4 f	32.4 e	36.0 e	36.9 e
	250	3.8 d	7.2 d	8.9 d	9.3 de	33.6 e	37.0 e	38.0 e
NH_4^+-enriched	10	1.7 e	2.7 f	3.3 f	3.4 g	9.3 f	11.4 f	12.3 f
	50	3.5 d	6.2 e	7.6 e	8.2 ef	37.0 de	42.8 d	44.2 d
	250	5.1 c	9.1 c	10.9 c	12.0 c	48.3 c	56.3 b	57.6 b
Water		7.1 b	12.7 b	19.0 b	21.0 b	61.2 b	71.5 a	72.5 a
$ZnCl_2$		25.8 a	47.7 a	61.4 a	68.4 a	70.5 a	71.8 a	72.5 a

[a] The values are means of three replicates. The data were transformed to ln-degree-asin ($x^{0.5}$) for statistical analysis. The values followed by the same letter(s) in a column within a run are not different according to the Fisher's least-significant-difference test at $\alpha = 0.05$.

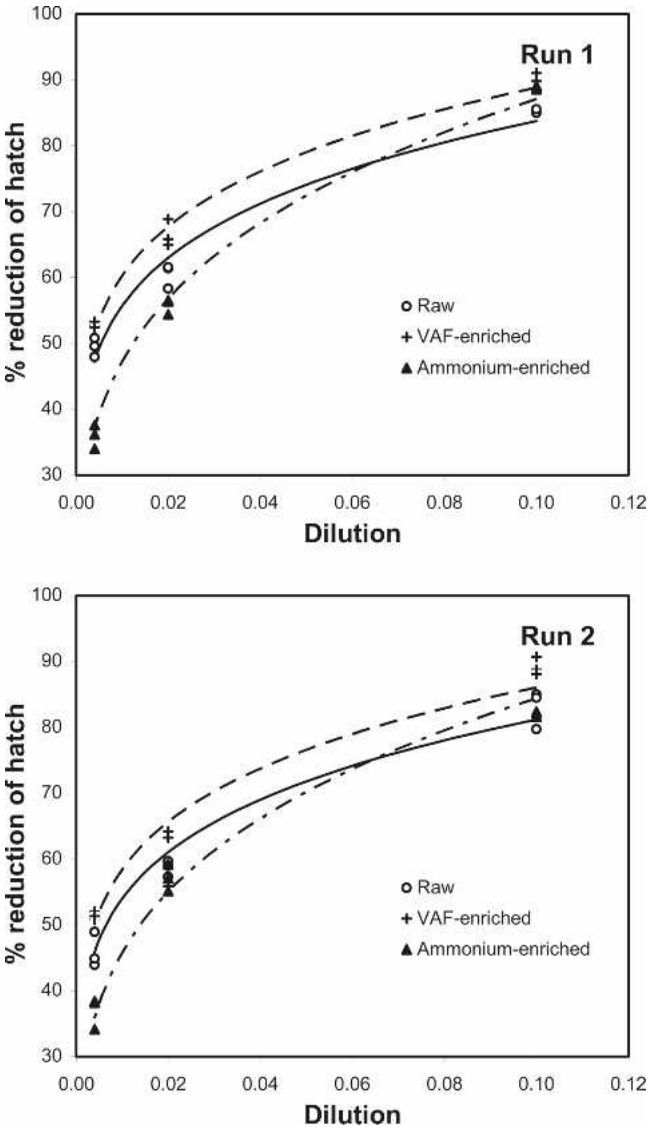

FIG. 1. Relationship between reduction of accumulated hatch of *Heterodera glycines* at day 21 and concentration of swine manure. The reduction of hatch was well predicted with the model: $\ln Y = 5.108 + 0.265X_1 - 0.242X_2 - 0.204X_3 - 0.031X_4 - 0.088X_1 \cdot X_2 - 0.097X_1 \cdot X_3$ ($R^2 = 0.977$, $P < 0.0001$), where Y = percentage reduction of hatch as compared with water control, $X_1 = \ln$(dilution of manure), $X_2 = 1$ if raw manure and 0 if others, $X_3 = 1$ if VFA-enriched manure and 0 if others, $X_4 = 1$ if Run 1 and 2 if Run 2.

tions at 8 hr than longer incubation time. At 8 hr, the J2 mortality in Run 1 was significant among all dilutions for the three types of manure, and the mortality increased with increasing concentration of manure. The trends were similar in Run 2 at 8 hr, although the morality between 4^{-1} and 16^{-1} dilutions was not significant. At 24 hr, J2 mortality between the two high concentrations (1 and 4^{-1} dilutions) became insignificant because most of J2 were killed at this point of time. The J2 mortality at 24 hr in the 64^{-1} dilution was still lower than the 16^{-1} dilution for raw and NH_4^+-enriched manures but not for VFA-enriched manure. At 48 hr, the J2 mortality at 16^{-1} dilution reached 91.7% to 100%

TABLE 3. Analysis of variance of percentage mortality of *Heterodera glycines* second-stage juveniles treated with swine manure filtrates.[a]

Source	*df*	*F* value 8 hours	24 hours	48 hours
		Run 1		
Rep	2	0.2	0.2	0.2
Manure	2	5.0*	34.0***	0.2
Dilution	3	727.6***	77.0***	116.4***
Manure*dilution	6	7.4***	20.2***	0.9
		Run 2		
Rep	2	0.3	2.1	2.9
Manure	2	0.4	24.8***	11.1***
Dilution	3	527.6***	98.6***	8.8***
Manure*dilution	6	1.1	9.5***	1.3

[a] The percentages of J2 paralyzed by the manure filtrate were degree-arcsine ($x^{0.5}$)-transformed before being subjected to analysis of variance. *df* stands for degrees of freedom. *, ** and *** represent significance at $P \leq 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

and did not differ from those at 1 and 4^{-1} dilutions in most cases. The mortality at 64^{-1} dilution ranged 81.6% to 84.2% in Run 1 and 90.2% to 95.3% in Run 2; the mortality was still lower than the other three higher dilutions in Run 1 for all three types of manure. The differences in J2 mortality among the three types of manure were observed at some dilutions and incuba-

TABLE 4. Percentage mortality of *Heterodera glycines* second-stage juveniles treated with swine manure filtrates.[a]

Incubation time (hr)	Dilution (x^{-1})	Manure Raw	VFA-enriched	NH_4^+-enriched
		Run 1		
8	64	4.9 d	2.0 d	5.6 c
8	16	9.6 cB	27.6 cA	8.2 cB
8	4	57.7 bA	50.4 bB	44.9 bB
8	1	95.3 a	98.7 a	98.0 a
24	64	64.6 cB	98.2 A	30.2 bC
24	16	93.5 bB	97.9 A	96.0 aAB
24	4	99.3 a	100.0	98.4 a
24	1	99.4 a	97.7	97.2 a
48	64	83.5 b	84.2 b	81.6 b
48	16	99.1 a	99.2 a	100.0 a
48	4	100.0 a	100.0 a	98.4 a
48	1	100.0 a	100.0 a	100.0 a
		Run 2		
8	64	0.8 d	1.5c	1.5 c
8	16	4.8 bc	7.5 b	9.7 b
8	4	13.3 b	12.8 b	15.9 b
8	1	98.3 a	95.3 a	95.8 a
24	64	63.1 cB	86.4 bA	49.0 cC
24	16	89.4 bA	93.4 abA	74.2 bB
24	4	92.1 b	98.3 a	97.4 a
24	1	96.9 a	97.9 a	98.4 a
48	64	92.2	95.3 b	90.2 b
48	16	91.8 B	99.3 aA	91.7 bB
48	4	93.5	97.5 ab	95.7 ab
48	1	97.4	99.1 a	98.3 a

[a] The values are means of three replicates. The data were transformed to degree-asin ($x^{0.5}$) for statistical analysis. The values followed by the same lowercase letter(s) or without letter in a column within an incubation time in a run or the values followed by the same uppercase letter in a row are not different according to the Fisher's least-significant-difference test at $\alpha = 0.05$.

tion times (Table 4). At 24 hr and 64^{-1} dilution, the mortality was highest for VFA-enriched manure, intermediate for raw manure, and lowest for NH_4^+-enriched manure in both runs, indicating the order of the three types of manure in terms of their nematicidal activity.

Effects on J2 infectivity: The infectivity was measured as the number of J2 that penetrated soybean roots in soil 48 hr after inoculation. The data of the two runs were similar, and thus were combined (Fig. 2). Only the 10^{-1} dilution of VFA-enriched and raw manures resulted in lower J2 number in the roots as compared with some low concentrations of the three types of manure. No statistical difference between the water control and any manure treatment was observed (Fig. 2).

Discussion

In spite of a number of studies showing that swine manure may have potential for control of microbial pathogens of plants (e.g., Conn and Lazarovits, 1999, 2000; Tenuta et al., 2002; Conn et al., 2005), there is limited research on swine manure for management of *H. glycines* (Reynolds et al., 1999; Stein et al., 2000; Xiao et al., 2007a). Our studies provide more insight on the value of liquid swine manure for disease and pest management.

In our previous greenhouse study, the *H. glycines* population density decreased with increased application rate of manure, and the VFA-enriched manure treatment was superior in suppression of egg population density at all application rates compared to other treatments (Xiao et al., 2007a). The present study showed the toxicity of the manures to *H. glycines* J2 and eggs. The VFA-enriched manure was the most, raw manure intermediate, and NH_4^+-enriched manure least effective in inhibiting *H. glycines* hatch and killing eggs and J2. The manure effect on *H. glycines* J2 hatch and viability is similar to its effectiveness in suppression of the nematode population in soil, indicating that main mechanism of the suppression of *H. glycines* population densities is probably the toxic effect on J2 hatch and mobility in soil.

The limited effect of the manure on the number of J2 that penetrated the soybean roots was unexpected. This was probably due to the methodology of the infectivity assay. During in vitro testing, the manures at 16^{-1} dilutions had little effect on J2 mobility within 8 hours. We treated the J2 only for 4 hours, which might not have been long enough to kill the J2 even at the highest concentration of 10^{-1} dilution. After adding the J2 along with the manure to soil, we watered the soil after 12 hours and once every 12 hours thereafter. It is possible that the addition of water diluted the concentration of manures and/or the soil reacted with the compounds in the manure, resulting in recovery for J2.

The interaction between manure type and dilution in affecting J2 hatch and viability indicates that the nematode responses to the doses of active compounds in different manures differ. VFA and ammonia are the two constituents in swine manure which have nematicidal properties. The VFA-enriched manure reduced J2 hatch and increased J2 mortality as compared with the NH_4^+-enriched manure, and the difference between the two manures was greater at lower manure concentration than higher concentrations, suggesting that VFA may be still effective at a relatively low concentration. In contrast, at the pH levels used in this study, the concentrations of ammonia are probably too low to be effective in control of *H. glycines*. While VFA and ammonia are the nematicidal compounds that are focused

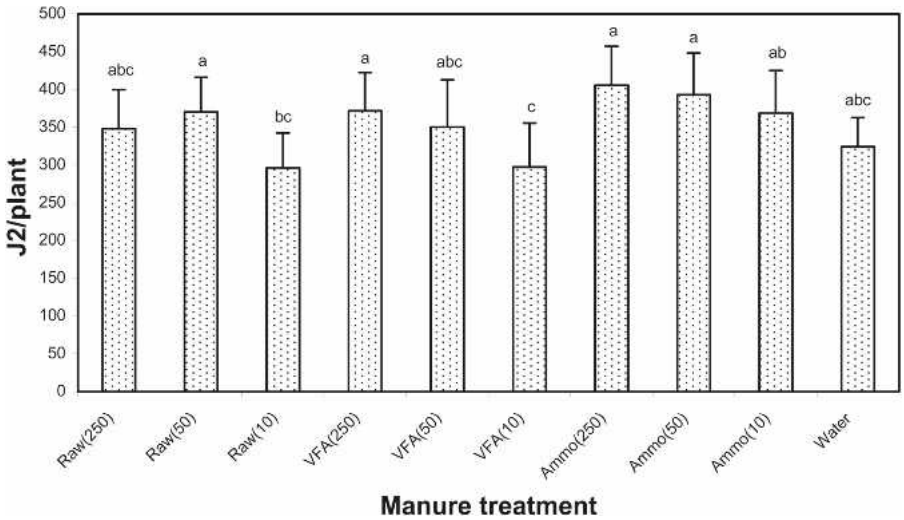

Fig. 2. Number of *Heterodera glycines* J2 penetrated per plant 48 hours after inoculation following the treatments of raw (Raw), VFA-enriched (VFA), or NH_4^+-enriched (Ammo) liquid swine manure. The numbers in the parentheses indicate dilution (x^{-1}) of the manures. Bars represent means and the lines above the bars are standard error of 10 replicates. The data were transformed to ln (x) values to improve homogeneity before being subjected to analysis of variance. The same letters on the bars indicate no significant difference according to the Fisher's least-significant-difference test at $\alpha = 0.05$.

on in our studies, other compounds present in swine manure cannot be ruled out for their involvement in nematode suppression.

Although the three types of manure are designated as VFA-enriched, NH_4^+-enriched and raw, there was small difference in the VFA and NH_4^+ concentrations among the manures. Many factors affect the concentrations of VFA and NH_4^+ in liquid swine manure (Conn et al., 2007). The time for VFA and NH_4^+ to reach their peaks may be different for different manure sources, different batches of manures from the same source, and/or different environmental conditions. We assumed that manures collected at 15 and 35 days of digestion had the highest concentrations of VFA and NH_4^+, respectively, based on the data from an earlier study (Xiao et al., 2007a). However, the difference in VFA concentrations between the 15-day sample and 35-day sample was greater in the previous study than in this study. In the earlier study, the manures were digested at the laboratory scale in a 15-liter cylinder rather than in a 4.5-m^3 tank as in the present study. The size of containers used may cause different conditions for the anaerobic digestion and consequently the time for achieving the highest concentrations of VFA and NH_4^+.

In spite of the small difference in the VFA and NH_4^+ concentrations among the manures, the treatment effect was significant. This treatment effect appeared to be mainly due to the VFA compounds. In general, VFA in swine manure include acetic, propionic, butyric, valeric and caproic acids (Le et al., 2005); most VFA in swine manure are acetic acid (60%–70%) and probionic acid (10%–20%), and the remainders represent only 10% to 20% (Conn et al., 2005, 2007). The total concentrations of VFA in the manures used in this study are comparable to those in other studies (Conn et al., 2005, 2007). In the previous study, the manures with VFA concentration more than about 5,300 mg/liter at the application rate of 30% soil moisture with a soil moisture level of 10% effectively suppressed the germination of fungal pathogen *Verticillium dahliae* microsclorotia (Conn et al., 2005). Different species of VFA may have different efficacy in killing microorganisms and nematodes. For nematode control, butyric acid, a main odor contributor, had the highest nematicidal activity in a previous study (McElderry et al., 2005). It may be worthwhile to further study the techniques to increase VFA concentration, especially the most effective VFA species, in swine manure for nematode management.

The concentration of ammonium in the manure used in this study is also comparable to that in previous studies (Conn et al., 2005, 2007). The level of the nematicidal ammonia is greatly affected by pH. At high pH levels, soil amendment such as N-Viro Soil may release ammonia (NH_3) to kill nematodes (Zasada and Tenuta, 2004). Although our data indicate that ammonia played a less important role in inhibiting the hatch and viability of *H. glycines* J2, further study is needed to determine whether there are ways to obtain higher concentration of NH_4^+ in swine manure and whether effective control of nematodes can be achieved with the processed swine manure at high soil pH under field conditions.

Although numerous plant and animal materials have been shown to suppress microbial pathogens and nematodes in greenhouse studies, generally high application rates are needed for most of these materials in order to achieve a significant suppression of nematodes, and, in most cases, the application rates are unpractical for field use because high rates of application may cause environmental and soil nutritional problems (Karlen et al., 2004). Understanding the mechanism of the waste materials in suppression of nematodes may help reduce effective application rates. This study shows that VFA are probably the most important compounds in the swine manure in suppressing *H. glycines* population density. One way to reduce the application rate is to increase the concentration of VFA in swine manure through anaerobic digestion. The highest level of VFA can be achieved after 17 to 18 days of digestion. Although there was only an increase of about 10% VFA compared with the raw manure used in this study or about an 18% increase in a previous study (Xiao et al., 2007a), the concentration of VFA in the manure digested for 15 days was much higher (35%–60%) than that for 35 days. Other approaches to increase VFA in swine manure have not been extensively investigated. Feed types, age of pigs, pH and microbial communities in the manure could be possible important factors in VFA production. Further studies are needed to explore the possibility of other practical methods to increase VFA in manure for effective management of nematodes. For example, it will be interesting to see if VFA can be enhanced by adding or encouraging VFA-producing microorganisms, such as *Clostridium beijerinckii* (=*Clostridium butyricum*), which is a known, common VFA-producing bacterium in soil (McElderry et al., 2005).

In order to achieve effective suppression of *H. glycines* population density, appropriate application time needs to be considered. Based on the hatch assay in the laboratory, the swine manure can cause mortality of eggs, especially at high concentrations. If this mortality of eggs can be achieved in fields, the manure can be applied at any time to lower the nematode population density before planting soybean. However, the mortality of eggs may depend on the concentration of the manures. The effectiveness in killing *H. glycines* eggs needs to be evaluated under field conditions. Because the manure affects the hatch and viability of the J2, which is the infective stage, and VFA may play a major role, applying the manure with enriched VFA at planting appears to be a good practice to reduce application rate for effective management of *H. glycines*.

In conclusion, liquid swine manures digested anaerobically for different lengths of time have different effects on *H. glycines* J2 hatch, viability and infectivity. The manure with enriched VFA obtained at 15 days of anaerobic digestion showed the highest efficacy in inhibiting *H. glycines* J2 hatch and killing eggs and J2. The VFA are probably the major compounds in the manure responsible for the nematicidal activity. Enhancing VFA concentration with anaerobic digestion or any of other approaches and applying the manure at planting to suppress *H. glycines* J2 hatch and viability are probably the appropriate ways to achieve effective management of *H. glycines* at a realistic application rate in fields.

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