

# Chemical-Mediated Toxicity of N-Viro Soil to *Heterodera glycines* and *Meloidogyne incognita*<sup>1</sup>

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**Abstract:** N-Viro Soil (NVS) is an alkaline-stabilized municipal biosolid that has been shown to lower population densities and reduce egg hatch of *Heterodera glycines* and other plant-parasitic nematodes; but the mechanism(s) of nematode suppression of this soil amendment are unknown. This study sought to identify NVS-mediated changes in soil chemical properties and their impact upon *H. glycines* and *Meloidogyne incognita* mortality. N-Viro Soil was applied to sand in laboratory assays at 0.5%, 1.0%, 2.0%, and 3.0% dry w/w with a nonamended treatment as a control. Nematode mortality and changes in sand-assay chemical properties were determined 24 hours after incubation. Calculated lethal concentration (LC<sub>90</sub>) values were 1.4% w/w NVS for second-stage juveniles of both nematode species and 2.6 and >3.0% w/w NVS for eggs of *M. incognita* and *H. glycines*, respectively. Increasing rates of NVS were strongly correlated ( $r^2 = 0.84$ ) with higher sand solution pH levels. Sand solution pH levels and, to a lesser extent, the production of ammonia appeared to be the inorganic chemical-mediated factors responsible for killing plant-parasitic nematodes following amendment with NVS.

**Key words:** amendment, ammonia, biosolid, *Heterodera glycines*, *Meloidogyne incognita*, nematodes, pH.

Biosolids are the nutrient-rich, solid organic matter recovered from the treatment of domestic sewage in wastewater treatment facilities. After processing, these materials are increasingly being marketed as commercial fertilizers, soil conditioners, and landfill cover. Although agronomic benefits of biosolids as nutrient sources have been demonstrated (Basta, 2000; Sloan and Basta, 1995), there are concerns about pathogenic microorganisms and the mobility and availability of heavy metals. Technologies have been developed for the treatment of biosolids to yield a pathogen-free and stable product during storage and transportation. One such technology is the N-Viro process where digested municipal biosolids are mixed with alkaline reagents such as cement kiln dust, lime kiln dust, coal ash, or flue-gas de-sulfurization byproducts (Logan and Burnham, 1995). The final products, N-Viro Soils (NVS), are solid, granular materials with many desirable agronomic properties.

*Heterodera glycines*, the soybean cyst nematode, causes greater yield reduction in soybean (*Glycine max*) than any other pathogen or pest in the United States (Wrather et al., 2001). The most common control measures implemented are crop rotation and resistant cultivars. Although these are effective nematode management tools, both have their shortcomings (Koenning et al., 1993; Schmitt, 1991) and alternative *H. glycines* management options are needed.

In addition to the soil fertility and organic matter

benefits associated with NVS, another benefit may be plant-parasitic nematode suppression. Soybean fields in Ohio receiving an NVS application were discovered to have reduced populations of *H. glycines*. This reduction, which was sustained for 3 years in some fields, enabled soybeans to be inserted into a crop rotation cycle 2 to 3 years earlier than in a nonhost crop rotation cycle (pers. comm.). In greenhouse experiments NVS applied at rates equivalent to 2 to 20 dry t/ha reduced *H. glycines* populations. In parallel field experiments, a 2.2-dry t/ha NVS application rate did not reduce *H. glycines* populations (Welacky and Topp, 2001). Additional field and greenhouse trials with NVS have resulted in similar observations of variable success in reducing *H. glycines* and other plant-parasitic nematode populations (Koenning, Li, Melakeberhan, Riedel, Tylka, unpubl. data).

The goal of this study was to investigate possible chemical-mediated mode(s) of nematode suppression by NVS amendment. The specific objectives were to (i) compare a range of NVS application rates against second-stage juvenile (J2) and egg stages of *H. glycines* and *Meloidogyne incognita*, (ii) relate changes in sand solution chemical constituents after an NVS application to nematode mortality, and (iii) prioritize the most important chemical constituents responsible for nematode suppression.

## MATERIALS AND METHODS

**Nematode inoculum:** *Heterodera glycines* race 3 from Salisbury, Maryland, cultured on greenhouse-grown soybean cv. Essex was used throughout this study. Four-month-old plants were removed from their pots, soil adhering to the roots was collected, and the root system agitated in water for several minutes to dislodge females. Cysts were extracted using centrifugation and flotation (Jenkins, 1964) to provide eggs and J2 for subsequent experiments. For experiments involving eggs, cysts were crushed with a glass homogenizer and the resulting solution poured over nested 250- and 25-

Received for publication 19 December 2003.

<sup>1</sup> Mention of a trademark of a proprietary product does not constitute a guarantee, warranty, or endorsement by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other suitable products.

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The authors thank Paula Crowley and Steven Rogers (USDA-ARS Beltsville) and Bert Luit (University of Manitoba) for technical support, N-Viro International for providing the material used in these experiments, and David Chitwood (USDA-ARS Beltsville) and Haddish Melakeberhan (Michigan State University) for reviewing this manuscript.

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This paper was edited by P. A. Roberts.

$\mu\text{m}$ -pore sieves, with the eggs retained on the latter. To obtain J2, eggs were placed on a modified Baermann funnel and 24 hours later J2 were collected and used immediately in laboratory assays.

*Meloidogyne incognita* originally isolated from a field near Salisbury, Maryland and cultured on greenhouse-grown pepper (*Capsicum annuum*) cv. PA-136 was used in all experiments. Eggs were obtained from roots (Hussey and Barker, 1973) and either used directly in assays or placed on a Baermann funnel to hatch; 24 hours later J2 were collected and used immediately in laboratory assays.

*Assay system and amendment experiments:* All-purpose sand (Global Stone James River, Buchanan, VA) having a pH of 6.5 in water was used in all experiments. The sand was passed through an 850- $\mu\text{m}$ -pore sieve to obtain uniform particle size, washed in distilled water, and dried prior to use. An NVS amendment stabilized with fly ash (N-Viro International Corp., Toledo, OH) was applied at 0%, 0.5%, 1.0%, 2.0%, and 3.0% dry weight amendment/dry weight sand (% w/w), corresponding to field applications of 0, 10, 20, 40, and 60 dry t/ha furrow slice. The amendment was mixed into the sand at the selected rates prior to being loaded into the assay container. The NVS amendment used was highly alkaline with a pH reaction in water of 12.2, a total solids content of 75%, 160 kg/mg calcium, 53 kg/mg sulfur, 17 kg/mg magnesium, 10 kg/mg potassium, 4 kg/mg total nitrogen, 2.8 kg/mg phosphorus, and 0.3 kg/mg  $\text{NH}_4^+\text{-N}$  (analyses by Biocheck Laboratories, Toledo, OH).

Eight  $\text{cm}^3$  of sand mixture, with or without amendment, was placed into a 2.0  $\times$  2.9-cm polypropylene tube assay assembly similar to that described by Behm et al. (1995) (Fig. 1). The tube was made of three components. The outer ring was a 2.1  $\times$  2.9-cm piece with a 0.5  $\times$  0.3-cm half-circle notch at the base. The inner ring was 1.9  $\times$  1.6 cm. A piece of 25- $\mu\text{m}$ -pore polyester mesh, 4.5 cm diam., was placed over one end of the inner ring and then inserted into the outer ring until

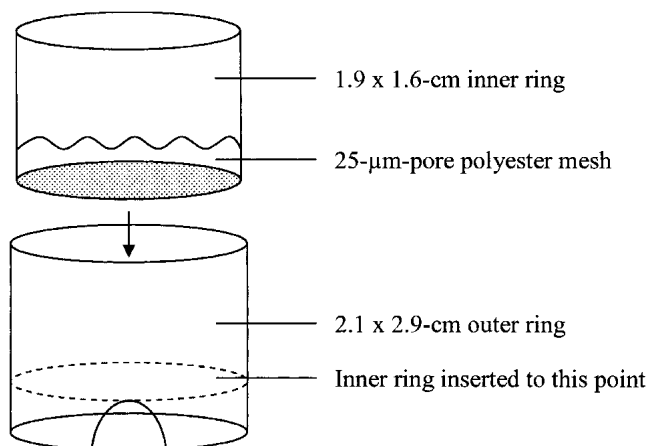


FIG. 1. Assay system used in N-Viro Soil laboratory experiments.

the mesh was flush with the top of the half-circle notch. After placing the sand mixture in the tube, it was gently tapped on a bench to allow the sand to settle. Then, 250 to 300 J2 or 500 eggs were added to the top of the sand in 700  $\mu\text{l}$  water, bringing the moisture content of the sand mixture to approximately 60% of water-filled-pore space. The tubes were placed in a covered 5-cm-diam. petri dish and incubated at 25 °C in the dark for 24 hours.

The tubes were placed in 5.0  $\times$  1.5-cm counting dishes to recover surviving J2 nematodes. Tap water was added to the dishes until water just touched the bottom of the mesh (above the top of the half-circle notch at the base of the tube assembly). The recovery units were covered with a glass dish to prevent water loss. Nematodes from tubes inoculated with J2 were extracted for 48 hours; in egg-containing tubes J2 were allowed to hatch from eggs for 1 week. Thereafter, the tubes were removed, and the number of J2 that moved through the mesh was quantified directly in the counting dish using a dissecting microscope. Treatments were replicated four times, and each nematode and or life stage experiment was conducted two times.

*Chemical measurements:* An identical set of tubes was prepared, without nematodes, to measure changes in the chemical characteristics of the sand solution after amendment with NVS. After the 24-hour incubation period, 1:5 sand:water mixtures were prepared from the amended and nonamended sand. The mixtures were placed on a shaker for 15 minutes and allowed to settle for 15 minutes; the pH of the solutions was then determined.

For ammonium plus ammonia analysis, 1 ml of the 1:5 sand:water supernatant was removed, placed into a 1.5-ml micro-centrifuge tube, and centrifuged at 1,600 g for 5 minutes. The supernatant was removed and filtered through a 0.22- $\mu\text{m}$ -pore filter. The ammonia plus ammonium concentration of the filtered sand solution was measured using a colorimetric method based upon the Berthelot reaction (Rhine et al., 1998). Ammonia concentration in sand solution was estimated using the Henderson-Hasselbach equation describing the pH-dependent equilibrium between ammonium and ammonia (Tenuta and Lazarovits, 2002b). Chemical constituent concentrations in sand solution were analyzed by Inductively Coupled Plasma spectroscopy (ICP) (Agricultural Analytical Services Laboratory, Pennsylvania State University, University Park, PA). Fifteen ml of the sand solution was filtered through a #541 Whatman filter (Kent, UK) prior to ICP analysis. All chemical constituent concentrations were expressed as mM. The presence of specific cations and anions in sand solution was determined on one set of samples by ion chromatography.

*Addition of chemical constituents of assay solution:* Potassium sulfate ( $\text{K}_2\text{SO}_4$ ) and calcium chloride ( $\text{CaCl}_2$ ) (Sigma Chemical Co., St. Louis, MO) were tested

against J2 of *H. glycines* and *M. incognita* to evaluate the lethality of these specific chemical constituents of NVS amended sand. Inductively Coupled Plasma analysis showed S and Ca to be dominant constituents of NVS-amended sand solution, and ion chromatography analysis identified them to be in the forms of  $\text{SO}_4^{2-}$  and  $\text{Ca}^{2+}$ . Both compounds were applied at rates encompassing concentrations measured in sand solution after the application of NVS. Nematodes were exposed for 24 hours to concentrations ranging from 25 to 100 mM in the sand assay system described above.

Ammonia, initially in the form of ammonium chloride ( $\text{NH}_4\text{Cl}$ ), was tested at final concentrations ranging from 0.2 to 1.8 mM. Dilutions of  $\text{NH}_4\text{Cl}$  were prepared in glycine buffer, pH 8.6 (Tenuta and Lazarovits, 2002a). A buffer-only control was included. To determine the influence of pH on nematode mortality, calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) was added to generate sand solutions ranging in pH from approximately 9 to 11. For assays testing ammonia and  $\text{Ca}(\text{OH})_2$ , the systems were closed with tight-fitting rubber stoppers to limit the loss of ammonia and to minimize acidification of the assay solution from absorption of atmospheric  $\text{CO}_2$  into  $\text{Ca}(\text{OH})_2$  solution. pH was measured 3 hours after  $\text{Ca}(\text{OH})_2$  application, this being the point of highest sand solution pH. Each treatment was replicated four times and the experiments conducted two times.

**Statistical analyses:** All nematode mortality data were expressed as a percentage decrease of the number of nematodes surviving in nonamended or nontreated controls. For NVS—nematode dose response curves, data for each replicate were arcsine-transformed and subjected to linear least-squares regression analysis. The fitted models were appropriate, with the Shapiro-Wilk goodness-of-fit for every data set being  $w > 0.10$  at  $P > 0.01$ . The toxicity of NVS against nematodes was calculated as the amount that caused 90% nematode mortality ( $\text{LC}_{90}$ ). These results are presented as the average  $\text{LC}_{90}$  ( $\pm 95\%$  confidence interval) of the combined experiments. The relationship between pH and chemical constituents measured in sand solution after NVS amendment to nematode mortality was also determined using linear least-squares regression models. Nematode mortality data were arcsine-transformed when necessary to meet the assumptions of the model. pH—nematode mortality slopes for NVS and  $\text{Ca}(\text{OH})_2$  were log-transformed and the means compared with Student's *t*-Test ( $P < 0.05$ ). All data were analyzed using the computer software JMP (SAS Institute, Cary, NC).

## RESULTS

The percentage recovery of nematodes in the nonamended or nontreated controls compared to the number of nematodes added was 80% for J2 of both nematode species, 75% for *M. incognita* eggs, and 55% for *H. glycines* eggs (data not shown). The survival response

curves for *H. glycines* (Fig. 2A) and *M. incognita* (Fig. 2B) were similar for the tested life stages. For each species, the J2 were more susceptible to NVS than eggs, with 100% J2 mortality occurring at NVS rates  $\geq 2.0\%$ . This level of mortality was not achieved for nematode eggs regardless of the NVS rate tested. The responses of the J2 and egg stages of both nematode species resulted in similar  $\text{LC}_{90}$  values. For *H. glycines* and *M. incognita* J2, the  $\text{LC}_{90}$  value was 1.4% (1.3 to 1.6) NVS. In contrast, eggs had higher  $\text{LC}_{90}$  values of  $>3.0$  (2.4 to  $>3.0$ ) and 2.6% (2.3 to 2.8) NVS for *H. glycines* and *M. incognita*, respectively. The  $\text{LC}_{90}$  values were not different between nematode species.

Compared to other measured chemical components, the concentration of calcium measured in sand solution was most closely related to nematode mortality, with coefficients of determination of fitted least-squares regression models ( $r^2$ ) ranging from 0.83 to 0.95 (Table 1). At the highest application rate of NVS, the nematodes were exposed to 79.7 mM of calcium in sand solution (Table 2). Sulfur concentration and the pH of the sand solution also were strongly correlated with nematode mortality. The concentration of sulfur was the next-highest measured chemical constituent of the sand solution to which nematodes were exposed, ranging from 24.2 to 42.5 mM at NVS application rates of 0.5 and 2.0%, respectively. The pH of the sand solution after 24 hours ranged from 7.3 to 11.2 as the rate of

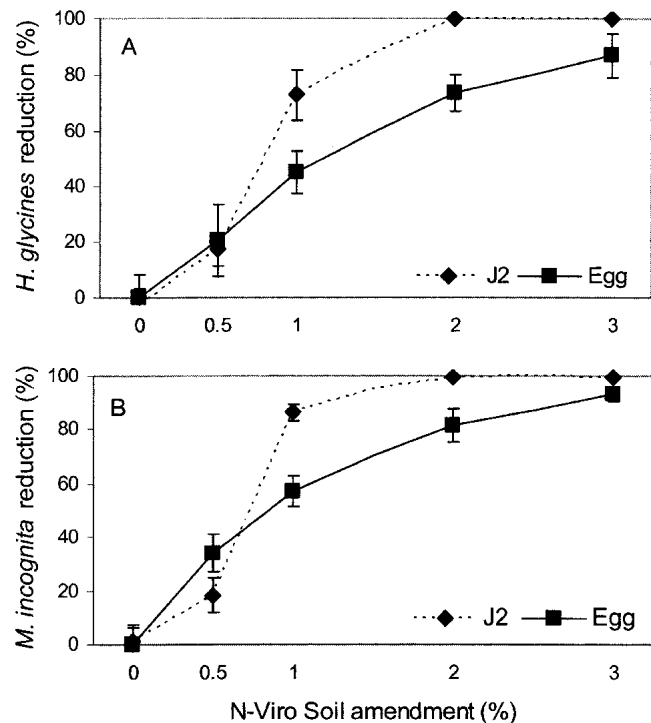


FIG. 2. Response curves for *Heterodera glycines* (A) and *Meloidogyne incognita* (B) second-stage juveniles (J2) and eggs exposed to a range of N-Viro Soil application rates. Nematode mortality was determined after a 24-hour exposure period in a sand assay system. The values are the average ( $\pm$ SD) of two experiments with four replications for each treatment.

TABLE 1. Coefficients of determination ( $r^2$ ) for linear models describing the relationship between nematode mortality and the concentration of chemical constituents in sand solution after N-Viro Soil amendment.

Constituent	<i>Heterodera glycines</i>		<i>Meloidogyne incognita</i>	
	Juveniles	Eggs	Juveniles	Eggs
Ca	0.86	0.90	0.83	0.95
Mg	NR	NR	NR	NR
S	0.81	0.73	0.80	0.87
K	NR	NR	NR	NR
Na	0.40	0.58	0.42	0.57
NH <sub>3</sub>	0.45	0.54	0.56	0.69
pH	0.72	0.77	0.62	0.81

Only fitted models having an overall significance of  $P < 0.05$  are given; all others are given as NR = no relationship.

NVS increased from 0 to 3.0% (Table 2). Ammonia and sodium concentrations were moderately related to nematode mortality, with  $r^2$  values ranging from 0.40 to 0.69 (Table 1). The highest concentration of ammonia measured after 24 hours was 1.0 mM at a 3.0% NVS rate (Table 2). There was no relationship between the concentrations of magnesium and potassium measured in sand solution and nematode mortality.

K<sub>2</sub>SO<sub>4</sub> and CaCl<sub>2</sub> were not lethal to J2 of *H. glycines* or *M. incognita* at concentrations of 25 to 100 mM (data not shown). Ca(OH)<sub>2</sub>, applied to achieve a range of soil pH levels as measured after NVS amendment, was strongly related ( $r^2 = 0.84$ ) to *H. glycines* J2 mortality (Fig. 3). The slope of linear least-squares regression models for *H. glycines* survival for pH after NVS amendment and pH after Ca(OH)<sub>2</sub> application were similar ( $P > 0.05$ ). The generation of ammonia from NH<sub>4</sub>Cl in sand resulted in lower mortality of *H. glycines* J2 than comparable ammonia levels observed following NVS amendment. None of the concentrations of ammonia to which nematodes were exposed (0.2 to 1.8 mM) resulted in 100% mortality. The highest exposure concentration of ammonia tested resulted in *H. glycines* J2 mortality of 89% ( $\pm 3$ ) compared to the nontreated control. The second-highest concentration of ammonia

TABLE 2. Sand solution pH and concentration (mM) of chemical constituents 24 hours after the application of N-Viro Soil.<sup>a</sup>

	N-Viro Soil Addition (% w/w)				
	0	0.5	1.0	2.0	3.0
pH	7.3 ( $\pm 0.2$ ) <sup>b</sup>	9.9 ( $\pm 0.2$ )	10.3 ( $\pm 0.1$ )	10.8 ( $\pm 0.2$ )	11.2 ( $\pm 0.1$ )
Ca	0.5 ( $\pm 0.3$ )	32.4 ( $\pm 6.9$ )	46.5 ( $\pm 4.1$ )	66.6 ( $\pm 4.0$ )	78.7 ( $\pm 3.0$ )
Mg	0.3 ( $\pm 0.2$ )	0.7 ( $\pm 0.2$ )	0.8 ( $\pm 0.1$ )	0.4 ( $\pm 0.2$ )	0.3 ( $\pm 0.1$ )
S	1.9 ( $\pm 1.1$ )	24.2 ( $\pm 4.5$ )	34.4 ( $\pm 2.2$ )	42.5 ( $\pm 4.1$ )	40.5 ( $\pm 4.0$ )
K	1.5 ( $\pm 1.1$ )	1.6 ( $\pm 0.7$ )	1.6 ( $\pm 0.8$ )	1.7 ( $\pm 0.3$ )	2.1 ( $\pm 0.3$ )
Na	1.1 ( $\pm 0.3$ )	1.3 ( $\pm 0.1$ )	1.4 ( $\pm 0.2$ )	1.6 ( $\pm 0.2$ )	1.7 ( $\pm 0.3$ )
NH <sub>3</sub>	0.0	0.8 ( $\pm 0.1$ )	0.8 ( $\pm 0.1$ )	0.8 ( $\pm 0.1$ )	1.0 ( $\pm 0.1$ )

<sup>a</sup> Other than pH and NH<sub>3</sub>, constituents were measured by inductively coupled plasma spectroscopy. NH<sub>3</sub> was estimated using Henderson-Hasselbach equation.

<sup>b</sup> Values shown are the average of two experiments with four replications for each treatment ( $n = 8 \pm 1$  SD).

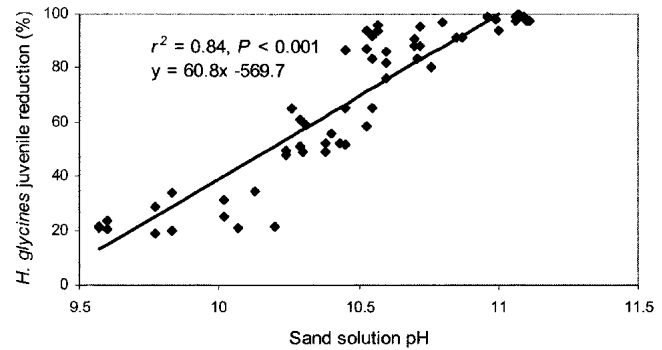


FIG. 3. Response curve for second-stage juveniles of *Heterodera glycines* exposed to Ca(OH)<sub>2</sub> resulting in a range of sand assay solution pH levels. Juvenile mortality was determined after a 3-hour exposure period. Sand solution pH was determined on the supernatant of a 1:5 sand:water mixture. The values are the average of two experiments with four replications for each concentration of Ca(OH)<sub>2</sub>.

measured, 0.9 mM, resulted in a 21% ( $\pm 13$ ) reduction in nematode survival (data not shown).

## DISCUSSION

To use organic amendments successfully for the management of plant-parasitic nematodes, it is necessary to understand the mechanism(s) of nematode suppression associated with the amendment. Without this information, it is difficult to anticipate or generalize about the performance of specific amendments (McSorley et al., 1997). This research aimed to determine relationships between changes in inorganic chemical constituents of sand amended with NVS to mortality of plant-parasitic nematodes.

N-Viro Soil suppressed the survival of J2 and eggs of *H. glycines* and *M. incognita* at hectare equivalent rates ranging from 10 to 60 dry t/ha. *Heterodera glycines* was suppressed by NVS in other studies at application rates of 2 to 20 dry t/ha (Welacky and Topp, 2001) and 10 and 55 t/ha (Alptekin, 2001). Other biosolid materials have been evaluated for plant-parasitic nematode suppression. Amendment of field soil with heat-treated sewage sludge (Mannion et al., 1994) or dried, pelletized biosolids (McSorley et al., 1997) did not provide consistent plant-parasitic nematode suppression. While a significant difference in galling was reported between biosolid-amended and nonamended pots, roots were still severely galled in biosolid-amended treatments (Castagnone-Sereno and Kermarrec, 1991).

Alkaline stabilization and low water content differentiate NVS from most other biosolid amendments. To some extent, NVS has similar chemical characteristics to a high-organic-matter, saline, calcareous soil (Logan and Harrison, 1995). Calcium dominates the chemical characteristics of NVS (Yamakawa, 1999). Because of its alkalinity, NVS is often marketed as a substitute to liming agents. N-Viro Soil can be applied to land at a cost of approximately \$13/t/ha to achieve the same results as a \$38/t/ha lime application.

Our assay system was able to identify that the generation of high pH following NVS amendment was a major factor responsible for acute nematode mortality. *Heterodera glycines* J2 mortality was comparable at similar sand solution pH levels generated after NVS amendment and after  $\text{Ca}(\text{OH})_2$  application. Because pH controls key reactions for many elements including adsorption-desorption, precipitation-dissolution, and complexation with organic matter, it is an important factor that can affect the chemical characteristics of NVS. In a water mixture, NVS has a high initial pH of 12.2. When NVS was incorporated into our sand assay of low buffering ability, the initial rapid  $\text{Ca}(\text{OH})_2$ -induced increase in soil pH was probably buffered by dissolution of atmospheric  $\text{CO}_2$  resulting in carbonic acid ( $\text{H}_2\text{CO}_3$ ) production and then precipitation of calcium carbonate ( $\text{CaCO}_3$ ), ultimately stabilizing sand solution pH.

We attempted to expose nematodes to the dominant inorganic chemical constituents of NVS. We applied calcium and sulfur at concentrations similar to those measured in solution after NVS amendment. They were not lethal to nematodes at any concentration. Any relation to nematode mortality by these constituents was not causal but coincidental with NVS addition rate. In previous experiments salts of  $\text{Ca}^{2+}$  had no effect on J2 of *M. incognita* (LeSaux and Quénehervé, 2002) or *M. javanica* but attracted J2 of *Tylenchulus semipenetrans* (Abou-Setta and Duncan, 1998).  $\text{K}_2\text{SO}_4$  attracted *M. incognita* (LeSaux and Quénehervé, 2002). In our studies, there were only weak relationships between nematode mortality and the other chemical constituents measured in soil solution (magnesium, potassium, and sodium). Similarly, the influence of soil properties on *H. glycines* populations in the field showed no correlation with magnesium and an increase in disease severity with increasing concentrations of potassium (Schermer et al., 1998).

When *H. glycines* J2 were exposed to concentrations of ammonia ranging from 0.2 to 1.8 mM, almost complete mortality occurred only at the highest ammonia concentration. Of all the chemicals released from organic compounds through microbial activity that are detrimental to nematodes, ammonia has been the most widely studied (Stirling, 1991); free ammonia is nematicidal, whereas ammonium is not. The lethality of ammonia to organisms is attributed to its deleterious effect on the plasma membrane and its high pH (Rush and Lyda, 1982) and more recently to induced starvation of cells as they actively pump ammonium ions out of their cytoplasm into the environment (Britto et al., 2001). The exact nematicidal mechanism of ammonia is not known. Because the relative amounts of ammonium and ammonia are pH dependent, high concentrations of ammonia are more likely to occur in alkaline conditions ( $\text{pK}_a = 9.3$ ). Ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) had both the greatest pH value and the greatest nematicidal activity against *M. javanica* of several ammonium com-

pounds tested (Oka and Pivonia, 2002). *Meloidogyne incognita* J2 were strongly repelled by ammonium salts and ammonium nitrate (LeSaux and Quénehervé, 2002). While ammonia may have played a role in nematode suppression at the highest NVS application rate used in this study, the amount measured 24 hours after amendment was not solely responsible for the nematode mortality reported here.

The ability to identify soil properties conducive to nematode control by NVS is essential. In acid soils, calcium carbonate formed following NVS amendment should dissolve, thereby decreasing pH below 8.2 (Goh, pers. comm.). Thus, NVS amendment may be more practical to control plant-parasitic nematodes in acid or poorly buffered soils, where pH can quickly increase but will eventually drop. Conversely, it may be difficult to raise soil pH to levels toxic to nematodes in well-buffered soils. Generally, the quantity of organic amendments generating ammonia required for nematode control in alkaline soil is less than that in acidic soil (Rodríguez-Kábana et al., 1987). The same would hold true for soil having weak compared to strong buffering capacity.

The impact of the high soil pH resulting from NVS amendment on crop productivity must be considered. Optimal plant growth usually occurs between pH levels of 5.5 and 7.6, and pH levels above or below this can compromise the availability of phosphorus and micronutrients. As discussed previously, the ability of NVS to suppress nematodes may be optimal in acid soils that can eventually buffer the initial increase in pH.

Although our modified laboratory assay provides valuable information about acute lethality and the sensitivity of different nematode genera and life stages to amendments, the bioassay cannot be used to predict effective field application rates. For example, the low buffering capacity of this sand assay most likely resulted in higher pH levels after NVS amendment, and therefore greater nematode mortality, than would be obtained in soil having a stronger buffering capacity.

Our study demonstrated that *H. glycines* and *M. incognita* were equally sensitive to NVS and that a difference in sensitivity occurred between nematode life stages. Differences in susceptibility of life stages to control practices have been demonstrated for nematicides (Mojtahedi et al., 1991) and transplant mixes (Kokalis-Burelle et al., 2002). The egg is often one of the most resistant stages in the nematode life cycle, and its three-layer shell may confer protection from hostile changes in its surrounding environment (Bird and Bird, 1991; Wharton, 2002). The implication of this finding is that targeting the nematode eggs, which would require higher rates of NVS, may not be practical. Second-stage juveniles of *H. glycines* and *M. incognita* should be targeted with an NVS amendment.

We have identified that change in sand solution pH, and to a lesser extent ammonia accumulation, contrib-

uted to nematode mortality. We have considered only the impact of NVS on the inorganic chemical constituents in sand solution; the contributions of organic compounds and microbial communities to nematode mortality have not been considered. *Heterodera glycines* and *M. incognita* J2 were equally sensitive to NVS amendment, with LC<sub>90</sub> values determined for juveniles being 1.4% w/w; NVS LC<sub>90</sub> values for eggs of both nematodes were higher than those for J2. These results indicate that development of NVS to control plant-parasitic nematodes will be most successful if the juvenile stage of nematodes and acid soils of low pH buffering ability are targeted for amendment.

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