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Effects of Biosolid Soil Amendment on *Heterodera glycines* Populations

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Abstract: The high degree of parasitic variability in *Heterodera glycines* and its distribution in a wide range of soybean production systems present multiple challenges for management, which necessitate increased understanding of the biology of *H. glycines*. Soil amendments are being considered either as stand-alone and/or as part of integrated management approaches. A recycled municipal biosolid with nutrition supplement and liming qualities, N-Viro Soil (NVS) has potential as a multi-purpose soil amendment. In three greenhouse experiments, the effects of 0, 1.0 or 4.0 g NVS/100 cm^3 of sandy loam soil on three *H. glycines* populations (GN1, GN2 and GN3) were investigated on soybean grown for 557 ± 68 degree-days (base 10°C). The response of the three *H. glycines* populations to NVS treatment varied by experiment. The overall numbers of preadult stages and cysts generally decreased with increasing levels of NVS in all experiments, and the high rate was more effective than the low rate in suppressing *H. glycines* numbers. This suggests that the high NVS treatment can affect the three populations in the experimental soil type under controlled conditions. Field studies to determine efficacy of the soil amendment in a wide range of environments should be initiated.

Key words: biosolid amendment, *Glycine max*, *Heterodera glycines*, management, nematode development, soil amendment, soil type, soybean, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a yield-limiting pathogen of soybeans worldwide (Wrather et al., 2001a, 2001b). Since its discovery in North America, *H. glycines* has been found in almost all soybean-producing states in the US and in Ontario, Canada (Riggs, 2004). Nearly 700 *H. glycines*-resistant cultivars are available to soybean producers (Shannon et al., 2004; Shier, 2005). However, resistance management is seriously threatened by the presence of parasitic (genetic) variability that exists within *H. glycines* populations (Niblack et al., 2002). Hence, alternative *H. glycines* management options that stand alone or can be integrated with existing practices are needed (Schmitt et al., 2004). Soil amendments may provide either complimentary or alternative management options to resistance management. These, however, require understanding *H. glycines* adaptation to the ranges of soybean production systems and selecting the type of amendment.

Analysis of *H. glycines* population diversity is based primarily on development of females on a set of soybean differential hosts. However, the geographic distribution of *H. glycines* covers a wide range of soil conditions (e.g., moisture-holding capacity, pH, soil nutrients, and texture), production, and cropping systems, indicating that *H. glycines* is adapted to diverse biotic and abiotic environmental conditions. In order to develop either complimentary or alternative management options to resistance management, it is necessary to consider how *H. glycines* genetic variability influences management alternatives.

Most agricultural soils require some level of nutrition and pH adjustments which, in turn, are beneficial to alleviating nematode-induced stress (Bumb, 1995; Melakeberhan, 1997; Baligar et al., 2001). The potential exists for the application of waste and/or other soil amendments to protect plants from plant-parasitic nematodes and other plant pathogens while managing soil nutrition.

N-Viro Soil (N-Viro International Corporation, Toledo, OH), an alkaline-stabilized municipal biosolid product used as a soil amendment, is a promising nematode and nutrient management alternative that deserves consideration (Logan and Burnham, 1995). Positive attributes of NVS include: nutrient and pH adjustment qualities, widespread availability, relative ease of application, and detrimental effects on several important plant parasites (Yamakawa, 1999; Alptekin, 2001; Welacky and Topp, 2001; Koenning, 2004; Zasada and Tenuta, 2004a, 2004b; Zasada, 2005).

The overall goal of this project is to understand how *H. glycines* populations adapt to diverse environmental conditions and management alternatives. The objective reported herein is to determine how *H. glycines* populations respond to NVS-based soil amendment. The working hypothesis was that *H. glycines* populations with different levels of parasitic ability on soybean host differentials will respond similarly to soil amendment.

Materials and Methods

The effects of 0, 1.0 or 4.0 g NVS/100 cm^3 of soil (wet weight basis) on *H. glycines* populations GN1, GN2 and GN3 were tested in three greenhouse experiments conducted at diurnal cycles of 8 hr dark and 16 hr light. In Experiments 1 and 2, temperature was set at 25°C ± 2°C with photosynthetically active radiation of 300 to 350 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at canopy level (Melakeberhan, 1999). A temperature of 28°C ± 2°C and photosynthetically active radiation of 450 to 550 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at canopy level were used in Experiment 3. Experiments 1 and 2 were terminated at 37 d and Experiment 3 at 31 d after

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nematode inoculation. The termination dates correspond to 555 ± 74 and 558 ± 62 degree-days (DD, base 10°C), respectively, and enough heat units to initiate a second generation of *H. glycines* (Melton et al., 1986).

Steam-sterilized sandy loam soil (87% sand, 8% silt, 5% clay, pH 7.0) was used. The respective NVS-amended soil treatments were mixed in 50 liter volumes, homogenized for 3 min in a cement mixer (Melakeberhan et al., 1995), poured into black plastic bags, and stored in plastic garbage cans. The NVS-amended soil for Experiments 1 and 2 was from the same lot, and that for Experiment 3 was repeated in time. The NVS-amended soil was stored for 3 wk before soybean seedlings were transplanted.

Seeds of an *H. glycines*-susceptible soybean cultivar, 'DSR-221' (Dairy Land Seed Co., West Bend, WI), were mass-germinated in the respective NVS treatments, selected for uniformity, and transplanted at 5 to 7 d after germination into pots containing the respective NVS treatments. In Experiments 1 and 2, 500 cm^3 of soil in clay pots was used, and 300 cm^3 of soil in styrofoam cups was used in Experiment 3. Pots were watered to saturation 1 hr prior to transplanting seedlings and watered with tap water as needed thereafter.

Heterodera glycines populations GN1 and GN2 were obtained originally from North Carolina and GN3 from Illinois; they were classified as HG type 2, HG type 1.2, and HG type 0, respectively (Niblack et al., 2002). The cultures were maintained under greenhouse conditions for three generations before eggs were collected using the standard semi-automatic elutriation method (Byrd et al., 1976; Avendaño et al., 2003). An inoculum density of 10,000 eggs/500 cm^3 of soil mix (Experiments 1 and 2) and 1,200 eggs/300 cm^3 of soil mix (Experiment 3) of each population was applied as described by Melakeberhan and Dey (2003). Control treatments received water. Inoculum density was estimated from four 1-ml suspensions. In order to determine the developmental stages of the inoculum cohort, embryogenesis (differentiated or undifferentiated) was determined as illustrated by Zuckerman (1985). In Experiments 1 and 2, the inoculum consisted of 60%, 64%, and 66% differentiated for populations GN1, GN2, and GN3, respectively. In experiment 3, the inoculum consisted of 30%, 24%, and 23% differentiated for populations GN1, GN2, and GN3, respectively. The 36 experimental units (pots), consisting of three levels of NVS (0, 1.0, and 4.0 g/100 cm^3 soil), a control minus nematodes and three populations of *H. glycines* populations (GN1, GN2, and GN3), were arranged in a completely randomized experimental design on greenhouse benches.

At the end of each experiment, pots were inverted to remove soil, and the roots gently separated from most of the soil to minimize dislodgement of cysts from the roots. Roots were placed in white plastic bags; cysts remaining in the soil were washed from the roots and extracted following standard laboratory procedures (Jenkins, 1964; Avendaño et al., 2003). The root system was then weighed, and a 2-g sub-sample randomly collected and stained in acid-fuchsin (Hussey, 1985) to determine *H. glycines* developmental stages. Stained root samples were kept at 4°C until counted. *Heterodera glycines* developmental stages were determined as illustrated by Agrios (1997) and Melton et al. (1986) and categorized as infective second-stage (J2), third/fourth-stage juveniles (J3 and J4), and males and females (Melakeberhan and Dey, 2003). Females included adults that were beginning to produce but had not laid eggs (Agrios, 1997). In this paper, the juveniles and females inside the root system will be referred as 'life stages' and those outside the root system as 'cysts.'

Data analysis: Nematode numbers were standardized on a per gram fresh root weight basis. Data were transformed to $\log_{10}(x + 1.5)$ before being subjected to statistical analysis using the PROC MIXED procedure (SAS, Cary, NC). Differences of Least Square Means was used to compare life stages recovered for different rates of NVS and among populations for numbers of life stages and cysts (SAS Institute Inc., Cary, NC; Steel and Torrie, 1980). When interactions occurred between NVS and *H. glycines* populations, differences among Least Square Means were compared. Due to recovery of life stages in only two samples in the 4.0 g NVS/100 cm^3 in Experiment 1, only the NVS 0 and 1.0 treatments were analyzed. Since variances of all experiments were not homogeneous, each experiment was analyzed separately.

Results

Significant effects of NVS on *H. glycines* life stages and cysts were observed, but they varied among the three experiments (Table 1). In Experiment 1, the NVS 4.0 treatment was highly detrimental to all life stages. Only two of 12 roots systems contained any life stages, but the means for each population are reported as 0. The large number of samples without nematodes precluded statistical analysis. In Experiments 2 and 3, numbers of life stages were lower for the 4.0 treatment than for the control and 1.0 treatment ($P = 0.05$). In Experiments 1 and 3, no differences ($P = 0.05$) were observed between the control and the 1.0 treatment. In Experiment 2, there were fewer ($P = 0.05$) life stages recovered from roots grown in the NVS 4.0 treatment when compared to the control and 1.0 treatment of NVS. However, a greater number of life stages ($P = 0.05$) were recovered from the 1.0 treatment of NVS than from the control. In the three experiments, no *H. glycines* population had consistently higher or lower numbers of life stages recovered per gram of root. Numbers of nematodes recovered were lower in Experiment 1 than in Experiments 2 and 3.

When the numbers of cysts were expressed as a percent of total population density, there was no clear relationship with NVS treatment (data not shown). In Experiments 1 and 2, numbers of cysts recovered were

TABLE 1. Mean numbers of life stages and cysts for three *Heterodera glycines* populations (Pop) per gram fresh root weight in soil treated or not treated with N-Viro Soil® (NVS) in three experiments (Exp)[a].

		Life stages[b]				Cysts			
		NVS (g/100 cm^3 soil)[c]				NVS (g/100 cm^3 soil)[d]			
Exp	Pop	0	1.0	4.0	Means	0	1.0	4.0	Means
1	GN1	3.1	0.2	0.0	1.6**A**	30.4	8.3	3.9	14.2**A**
	GN2	16.2	17.6	0.0	16.9**B**	97.8	33.0	10.7	47.2**B**
	GN3	17.3	2.8	0.0	10.0**AB**	283.3	100.3	11.0	131.4**C**
	Means	12.2	6.9	0.0		137.2**a**	47.2**b**	8.5**c**	
2	GN1	27.4	32.0	4.2	21.2**A**	25.2	25.3	17.0	22.5**A**
	GN2	99.3	359.8	38.7	166.0**B**	101.3	94.3	58.0	84.5**B**
	GN3	55.4	199.5	42.4	99.1**B**	525.8	206.3	115.8	282.6**C**
	Means	60.7**b**	197.1**a**	28.4**c**		217.4**a**	108.6**b**	63.6**c**	
3	GN1	100.1	76.2	32.2	69.5**A**	47.2**A**	27.6**aA**	15.2**bB**	30.0
	GN2	35.5	47.7	27.7	37.0**B**	64.7**A**	39.4**aAB**	31.6**aB**	45.2
	GN3	70.0	43.4	18.1	43.8**B**	50.4**A**	9.2**bC**	20.6**abB**	28.3
	Means	68.5**a**	55.8**a**	26.0**b**		54.1	26.9	22.4	

[a] Values are the mean of four replications for each experiment.
[b] Life stages include second, third, and fourth stage juveniles and females that were beginning to produce but not laying eggs inside the root system.
[c] When no interaction occurred, different lower-case letters indicate a significant difference among levels of NVS, and different upper-case letters indicate a significant difference among populations according to the *t*-test ($P \leq 0.05$).
[d] For the interaction of populations and NVS, a different lower-case letter indicates a significant difference among populations within a level of NVS, and a different upper-case letter indicates significant difference within populations across all levels of NVS according to the *t*-test ($P \leq 0.05$).

greater ($P = 0.05$) for the control than NVS treatments and for the 1.0 treatment compared to the 4.0 treatment (Table 1). An interaction of NVS treatment with nematode populations was observed in Experiment 3 ($P = 0.05$). No differences in numbers of cysts were observed among the three populations in the control. The number of cysts was lower for GN3 than GN1 and GN2 in the NVS 1.0 treatment. Whereas with the NVS 4.0 treatment, more cysts of GN2 were recovered when compared to GN1 but not GN3. Numbers of cysts recovered from GN1 and GN3 did not differ ($P = 0.05$). Fewer ($P = 0.05$) cysts were recovered from the NVS 4.0 treatment than from the control for each of the three *H. glycines* populations in Experiment 3. For GN1 and GN3, the number of cysts recovered was lower for the NVS 4.0 treatment than the NVS 1.0 treatment. The NVS 1.0 treatment did not differ from the control for GN1 and GN2, however the number of cysts of GN3 recovered was lower ($P = 0.05$) than from the control. The numbers recovered from GN2 at the NVS 1.0 treatment also differed from the 4.0 treatment.

Discussion

The overall numbers of all life stages and cysts generally decreased with increasing NVS doses in all experiments. However, there was some variation in level of suppression by *H. glycines* population and NVS dose. Variability in the population densities recovered is to be expected, in part due to differences in reproductive potential among the *H. glycines* populations and in levels of inoculum used. The recovery of more nematodes, on a per inoculum basis, in Experiment 3 than in Experiments 1 and 2 suggests that there may have been less competition at low (Experiment 3) than at high inoculum level. The data show that suppression of nematode numbers was greater in the 4.0 g NVS/100 cm^3 treatment than in the 1.0 g NVS/100 cm^3 treatment. Moreover, suppression across the three *H. glycines* populations was consistent in the 4.0 g NVS/100 cm^3 treatment, but variable in the 1.0 g NVS/100 cm^3 treatment. This suggests that a blanket NVS effect against these *H. glycines* populations for the experimental soil type required a high dose.

Wide ranges of experimental conditions make comparisons among different studies difficult. However, variable responses to the NVS amendment have been reported because some effects of NVS application on soil are short-lived (Welacky and Topp, 2001). The variable levels of suppression in the 1.0 g NVS/100 cm^3 amendment observed in this study suggest that this NVS treatment may be too low to adversely impact the nematodes. That there was not the same degree of variability in the 4.0 g NVS/100 cm^3 treatment shows that this rate was sufficient to adversely affect the nematodes. The high and consistent level of nematode population density suppression in the 4.0 g NVS/100 cm^3 treatment, however, needs to be considered with plant health-related factors.

The 1.0 and 4.0 g NVS/100 cm^3 treatments roughly correspond to 20 and 80 tonne/ha under field conditions (Zasada and Tenuta, 2004b). It is possible that a given NVS treatment may not produce the same results in different soil types, nor for host and nematode combinations. What level of NVS to apply, therefore, needs to be weighed in relation to desired effects on *H. glycines* population densities and plant and other environmental factors.

The experimental temperature conditions were enough for a second generation of *H. glycines* to be well underway (Melton et al., 1986), a fact supported by the presence of life stages including infective second-stage

juveniles. The lack of clear relationship between NVS treatments and percentage cysts recovered suggests that NVS may not alter nematode development once infection takes place. Differences in the proportions of life stages among the experiments are probably related to variability in inoculum cohorts. The mode of action of NVS in influencing host-nematode interactions under the experimental conditions is unknown.

The threats from *H. glycines*' persistence as eggs in cysts and its genetic (parasitic) variability to soybean germplasm are major challenges to soybean production (Wang et al., 2000; Atibalentja et al., 2001; Niblack et al., 2002). Either complimentary and/or alternative management options based upon an understanding of *H. glycines'* parasitic variability will be needed to deal with these challenges. With nutritional, soil pH adjustment, and potential socio-ecological benefits, NVS has potential as a soil amendment management option for *H. glycines* and other nematodes (Welacky and Topp, 2001; Koenning, 2004). Exploiting this potential, however, depends upon understanding the mode of action of NVS on the host-nematode-soil environment continuum. Zasada (2005) and Zasada and Tenuta (2004a, 2004b) have documented that soil pH and soil chemistry effects of NVS are among the factors influencing the efficacy of NVS against *H. glycines* and/or *Meloidogyne incognita*. It is possible such soil physio-chemical activities may be confounding nematode response to NVS treatment. By describing the relationships among NVS and *H. glycines* populations under controlled conditions, this study provides additional information regarding the use of this amendment for *H. glycines* management.

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