

# Integrated approaches to understanding and managing *Meloidogyne hapla* populations' parasitic variability

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## Abstract

The pending loss of methyl bromide (MBr) and less understood nematode adaptation behaviour present significant challenges in managing *Meloidogyne hapla* in vegetable and nursery industries in temperate climates. This study examined the reproductive potential of populations of *M. hapla* from three nursery (Fields 1–3), one vegetable (Field 4) production system, and their response to a nutrition-based N-Viro Soil<sup>®</sup> (NVS, recycled municipal biosolid) amendment. Fields 1, 2, 3, and 4 had loamy sand, sandy, sandy, and muck soils with pHs of 7.15, 6.56, 7.43, and 6.30, respectively. The muck soil, never treated with MBr, had two–three times more nitrogen than the nursery fields. Measured by nematode community structure ecological indices, the muck soil had better nutrient availability and energy channels than loamy sand soil. In repeated experiments, the four *M. hapla* fields and a glasshouse (control) population were subjected to 0, 1, or 4 g NVS 100 cm<sup>-3</sup> of sandy loam soil using tomato at 28 ± 2 °C for 504 ± 56 degree-days. While both NVS doses significantly reduced population densities of all populations compared with the controls, the high dose was more suppressive than the low dose. Within the NVS-free controls, the population from muck soil had the lowest reproductive potential, which, in turn, was positively correlated with the soil pH from where the nematodes came. Difference in reproductive potential between the nursery and the muck soil populations suggests that nematode adaptation may need to be considered in identifying and in managing populations of *M. hapla*. Hence, understanding if and how MBr use and soil pH and nitrogen may affect nematode adaptation.

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## 1. Introduction

The gradual phasing out of methyl bromide (MBr), with few viable and sustainable alternatives, presents significant challenges in managing root-knot nematodes (*Meloidogyne* spp.) for many in-field and in-glasshouse vegetable and nursery production systems (Giannakou and Anastasiadis, 2005; LaMondia, 1995, 1999). The absence of resistant cultivars to the northern root-knot nematode (*Meloidogyne*

*hapla*) in temperate vegetable and nursery crops is particularly challenging. While the suitability of a management option may vary by commodity and region, developing sustainable soil amendment-based MBr alternatives is among the strategic priorities for managing soil-borne pests and diseases identified by many of the affected industries in temperate climates (Anonymous, 2006a, b). Among other things, addressing the strategic priority requires careful considerations of: (i) the complexity of the nematode (s) in question, (ii) the effects of past and current crop production system practices on nematode behaviour, and (iii) what management options are likely to be most viable in the prevailing production systems. Production system encompasses soil types, agricultural input, and eco-geographic influences.

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In addition to lacking a detailed *M. hapla* biological profile within vegetable and nursery production systems, *M. hapla* has complex and less understood genetics compared with other common root-knot nematode species (Chen et al., 2003; Liu and Williamson, 2004). For the most part, *M. hapla* (and other nematodes) identifications are based on morphometrics, host range, some levels of biochemical and molecular analysis, and/or combinations of one or more tools. There is little ecological adaptation basis for identifying nematodes. Therefore, an understanding of the relationship between nematode reproductive potential and/or pathogenicity and their living environment will be helpful to identifying nematode behaviour as well as in management decision making.

While varying by commodity and region, most modern and intensive crop production systems have been heavily dependent on agricultural inputs (herbicides, fungicides, insecticides, nematicides, and synthetic fertilizers, to mention a few) for the last five decades (Wickham et al., 1997). It has been estimated that about 79% of the approximately 63,000 chemical compounds that are commonly used by industry worldwide are pesticides (Wickham et al., 1997). In addition to agricultural chemicals, industrial pollutions such as acid rain are major factors that change soil chemistry. The impact of chemical and industrial population cocktails on the resident nematode behaviour is poorly understood. However, any organism that survives such complex of ecological changes in its environment has to be able to adapt to those conditions.

Since *M. hapla* continues to be a problem in production systems with a range of agricultural inputs, it is reasonable to assume that it has wide adaptation to multiple soil environments. From the management point of view, such an adaptation raises at least two critical questions: (1) Do populations of *M. hapla* from different production systems differ in their reproductive potential and/or pathogenicity? If they do, it will indicate that there may be ecological reasons to the differences, and further lead to understanding the possible basis to the differences in the response of the populations. (2) Do the populations of *M. hapla* differ in their response to potential MBr alternative management option(s)? If they do, the one-option-fits-all management approach will be challenged. For example, mustard-type biofumigants are among the environmentally friendly approaches shown to negatively affect nematodes to varying degrees (Bulluck et al., 2002; Hafez and Sundararaj, 2001a, b; Lauzier, 2002; McSorley et al., 1997, 1999; Riga et al., 2003; Wang et al., 2003). However, isothiocyanate volatiles, secondary metabolites of mustard-type biofumigants, have similar properties to Vapam, a commercial fumigant that has been in use for sometime (Tsao et al., 2002). While it is unknown how nematodes from years of Vapam-treated soils respond to mustard types of biofumigants or other soil amendments with similar modes of actions, changing a management option without understanding the reasons may not be economically feasible or environmentally sustainable.

Thus, an integrated understanding of the variability in response to a management practice will be helpful.

The soils in which food and fibre are produced are often structurally and nutritionally depleted, and require some degree of amendments (Baligar et al., 2001; Frink et al., 1999; Tillman, 1999). In addition to developing crops that are efficient nutrient users (Good et al., 2004), soil nutrient depletion can benefit from many organic amendments that are also being considered as sustainable nematode management options (McSorley, 1998). These include N-Viro Soil<sup>®</sup> (NVS), recycled municipal biosolid with nutrient and pH adjustment qualities, wide availability, and relative ease of application, ecological benefits (Logan and Burnham, 1995), and adverse effects on several plant-parasitic nematodes under field and under controlled conditions (Koenning, 2004; Melakeberhan and Noel, 2004; Welacky and Topp, 2001; Yamakawa, 1999; Zasada, 2005; Zasada and Tenuta, 2004a, b). Over 1 million tonnes of commercial NVS is produced annually and used as soil amendments in agriculture, horticulture, and land reclamation (Anonymous, 2005). If such an amendment is to be used in a sustainable way, its efficacy in relation to nematode reproductive potential and/or pathogenicity and nematode habitat need to be considered.

The goal of the project is to develop sustainable nematode management options in diversified agricultural production systems through understanding nematode parasitic variability. The objectives of this study were to: (i) characterize selected soil biotic and abiotic conditions associated with populations of *M. hapla* in selected nursery and vegetable production systems and (ii) determine how the populations of *M. hapla* from the production systems respond to NVS treatment. The general working hypothesis is that populations of *M. hapla* from different production systems differ in their reproductive potential (and hence pathogenicity) and in their response to NVS.

## 2. Materials and methods

### 2.1. Field studies

#### 2.1.1. Sampling and field background analysis

A field survey was conducted to characterize edaphic conditions in fields suspected to have *M. hapla* problems in the western lower peninsula of Michigan in 2003 and in 2004. In addition, growers were asked to provide a rough estimate on if and for how long the surveyed fields have been receiving nematicides, MBr, in particular, and other agricultural inputs. On 15 and 26 August 2003, eight nursery (five *Hosta* and one each of *Coreopsis*, *Artemisia*, and *Ajuga* spp. (commonly known as Fragrant Plantain-Lily, Whorled Tickseed, Wormwood, and Bugleweed, respectively) and four vegetable (celery, corn, carrot, and potato) fields were surveyed to determine the presence of *M. hapla* and its fungal parasites, *Hirsutella* spp., and bacterial parasites like *Pasteuria* spp. The nursery and the vegetable fields represented three and two farms, respec-

tively. In each of the 12 fields, plant roots with approximately 800 cm<sup>3</sup> of rhizosphere soil shovelled from 15 to 20 cm depth from four locations covering approximately 60 m<sup>2</sup> area. Based on limited field history and background analysis, three nurseries (Fields 1–3) and one vegetable (Field 4) field were selected for further sampling. On 27 July 2004, five root and five soil sample combinations were collected from the same 60 m<sup>2</sup> areas used in 2003 in the Fields 1, 2, and 4. Field 3 was not included because it had similar soil type as in Field 2 (Table 1). In order to get a more comprehensive understanding of the respective farms, additional 5, 20, and 20 samples were collected from adjacent fields to Fields 1, 2, and 4, respectively.

### 2.1.2. Analysis of populations of *M. hapla*

Nematodes were extracted from soil (Jenkins, 1964) and morphologically identified. Samples in which *M. hapla* was present along with galling were noted. Based on the presence of *M. hapla* and field background, sub-samples of approximately 100 cm<sup>3</sup> of soil from Fields 1–4 were potted and tomato (*Lycopersicon esculentum* L.) cv Rutgers seedlings planted to initiate cultures. Egg masses were collected and further sub-cultures developed for pathogenicity studies following standard glasshouse conditions (Hussey and Barker, 1973). Throughout the text, field numbers and populations of *M. hapla* from the respective fields will be the same.

### 2.1.3. Analysis of *Hirsutella* and *Pasteuria* parasitism

In 2003, sub-samples of approximately 200 cm<sup>3</sup> of soil per sample (a total of 48) were sent to the University of Minnesota for analysis of *Hirsutella* and *Pasteuria* parasitism of *M. hapla*. Second-stage juveniles (J2) of *M. hapla* were extracted with a sucrose flotation and centrifugation technique (Jenkins, 1964). Parasitism of the J2 by *Hirsutella* and/or *Pasteuria* was determined following the procedures described in Liu and Chen (2000). In 2004, 11 composite soil samples were analysed for *M. hapla* parasitism by *Hirsutella* and *Pasteuria*. Each of the composite samples came from the five samples from a field.

### 2.1.4. Analysis of soil texture and nutrients

Soil texture, pH and nutrients of 12 composite sub-samples were determined by the Michigan State University Soil Testing Laboratory (Melakeberhan, 1999). Each sub-sample came from the four fields where the nematodes were obtained and cultured. All but the samples for Field 3 came from 2004 samples.

### 2.1.5. Analysis of nematode community structure

Analysis of nematode community structure (NCS) of 12 composite sub-samples from Fields 1 and 4 was done as a means of rough estimation of soil biological activities among the fields. While cost limitations did not allow extended NCS analysis at all locations, the two fields represent distinct soil types within the study scope (Table 1). Half of the soil samples came from the 60 m<sup>2</sup> area where the experimental nematodes were isolated and the other half from outside (adjacent fields) in the respective farms. NCS analysis was done in Dr. D. Neher's laboratory at the University of Toledo (Neher and Campbell, 1996). Nematodes were identified to trophic groups (Yeates et al., 1993a) and were computed to reflect the composition of the nematode communities using five ecological indices: (i) richness, (ii) Hill's diversity ( $N1$ , Hill, 1973), (iii) summed ecological maturity ( $\Sigma MI$ , Yeates, 1994), (iv) the ratio of fungivores to total decomposers ( $F/(F+B)$ , Yeates et al., 1993b), and (v) Wasilewska's index as a ratio of fungivores plus bacterivores to herbivores ( $(F+B)/H$ , Wasilewska, 1994). The indices are to measure diversity (richness), proportion and abundance ( $N1$ ), level of disturbance ( $\Sigma MI$ ), and the ratios, decomposition and energy flows of the systems.

## 2.2. Glasshouse studies

### 2.2.1. Experimental design

The glasshouse experiments were designed to test the reproductive potential of the different *M. hapla* populations and their response to the soil amendment NVS treatments. Using Rutgers tomatoes, a glasshouse (control) and the four field populations of *M. hapla* under either zero (check), 1, or 4 g NVS 100 cm<sup>-3</sup> of soil treatment were

Table 1  
Information on the crops grown and methyl bromide (MBr) use provided by growers, and soil type, pH and NO<sub>3</sub>-N analysis of the four fields where the population of *Meloidogyne hapla* was isolated in Western Michigan in 2003

Field	Crop	Soil characteristics*			
		MBr <sup>†</sup>	Soil type	Soil pH	NO <sub>3</sub> -N (mg ha <sup>-1</sup> )
1	<i>Hosta halcyon</i>	Yes	Loamy sand	7.15b	29.6 × 10 <sup>6</sup> b
2	<i>Ajuga metallicacripa</i>	Yes	Sandy	6.56c	35.0 × 10 <sup>6</sup> b
3	<i>Hosta franccee</i>	Yes	Sandy	7.43a	55.6 × 10 <sup>6</sup> b
4	Celery	No	Muck	6.30d	124.3 × 10 <sup>6</sup> a

\*Determined by the Michigan State University Soil Testing Laboratory (Melakeberhan, 1999), data are means of three replications.

<sup>†</sup>All fields received variable amounts of non-fumigant nematicides, and use many agricultural inputs such as fertiliser, insecticides, etc.

studied in two sets of glasshouse experiments. In each of Experiments 1 and 2, a total of 54 experimental units (3 NVS  $\times$  6 nematode populations  $\times$  3 replications) were used. Experiments 1 and 2 were set up at the same time, but repeated in space in a glasshouse. In Experiment 3, repeated in time, the six nematode populations (including a check), and zero (check) and 4 g NVS 100 cm<sup>-3</sup> of soil and four replications (a total of 48 experimental units) were used. Treatments were arranged in a completely randomized design on glasshouse benches. All experiments were terminated at 28 d or 504  $\pm$  56 degree-days (DD, base 10 C) after nematode inoculation.

### 2.2.2. Soil amendment

Steam-sterilized sandy loam soil (87% sand, 8% silt, 5% clay, pH 7.0, and 28  $\times$  10<sup>6</sup>, 119  $\times$  10<sup>6</sup>, 56  $\times$  10<sup>6</sup>, 139  $\times$  10<sup>6</sup>, and 1386  $\times$  10<sup>6</sup> mg ha<sup>-1</sup> of NO<sub>3</sub>-N, P, K, Mg, and Ca, respectively) was used. The NVS product (Anonymous, 2006c) was supplied by N-Viro International Corporation, Toledo, OH. The respective NVS-amended soil treatments were mixed in 50 l volumes, homogenized for 3 min in a cement mixer, poured into black plastic bags, and stored in plastic garbage cans for 6 months before tomato seedlings were planted (Melakeberhan et al., 1995). The NVS-amended soil for Experiments 1 and 2 were from the same lot and that for Experiment 3 was repeated in time. Changes in soil chemistry due NVS treatment were not measured.

### 2.2.3. Growth conditions and plant materials

Glasshouse conditions were set at 28  $\pm$  2 °C with diurnal cycles of 8 h dark and 16 h light for all experiments with photosynthetically active radiation of 300–350  $\mu$ mol s<sup>-1</sup> m<sup>-2</sup> (Experiments 1 and 2) and of 450–550  $\mu$ mol s<sup>-1</sup> m<sup>-2</sup> (Experiment 3) at canopy level. In experiment 1, all seedlings were 2-week-old when transplanted, whereas 50% of those in Experiment 2 were 19-d old. Both experiments ran concurrently on different benches. In Experiment 3, seedlings were 2-week-old when transplanted into experimental soil. In Experiments 1 and 2, 500 cm<sup>3</sup> of soil and clay pots were used and 300 cm<sup>3</sup> of soil and Styrofoam cups in Experiment 3. The pots were randomly arranged on glasshouse benches. Each pot was watered to saturation 1 h prior to transplanting and watered with tap water only as needed thereafter.

### 2.2.4. Nematode inoculation

Eggs of the four field (1–4) and one glasshouse (5) populations of *M. hapla* were obtained following Hussey and Barker's (1973) bleach (5% NaOCl) method and inoculated at 3000 eggs per pot at 7 d (Experiments 1 and 2) and 4 d (Experiment 3) after transplanting into NVS-amended soil. The no-nematode controls received water. Inoculum population densities, from four 1-ml suspensions, and stages of embryogenesis (differentiated or undifferentiated), from four 100 random-egg counts, were determined per population of *M. hapla* as described in Melakeberhan and Dey (2003) and as illustrated by

Zuckerman (1985). In Experiments 1 and 2, the inocula consisted of 64  $\pm$  5% differentiated stage and 21  $\pm$  10% differentiated stage in Experiment 3. Knowing the stages of embryogenesis are important for accurate determination of nematode developmental stages after infection.

### 2.2.5. Nematode population estimation and measurements

The termination time of the experiments was designed to correspond with the completion of one generation (Insera et al., 1982; Melakeberhan and Dey, 2003). Roots were very carefully separated from soil, gently washed free of soil, and rated for root-knot galling indices on a 0 (no galling) to 5 (more than 75% of the root system galled) scale (Kinloch, 1990). In order to minimize experimental errors, whole root systems were stained and nematodes counted (Melakeberhan and Dey, 2003). Stained samples were kept at 4 °C until counted, *M. hapla* developmental stages determined as illustrated in Agrios (1997), and categorized as second-stage juveniles (J2), third- and fourth-stage males and females (J3/J4) (Melakeberhan and Dey, 2003).

### 2.3. Data analysis

Descriptive statistics for soil pH and nutrient and nematode community structure ecological indices were analysed by analysis of variance performed with SAS System Release 8 (SAS Institute, Cary, NC, 2000). Non-transformed nematode community indices are reported in the results, but the indices  $F/(F+B)$  and  $(F+B)/H$  were arc-sine of square-root and log transformed (respectively) prior to analysis to meet assumptions of normality. Nematode population densities were standardized on a per gram fresh root weight basis. To determine nematode suppression by NVS and to equalize comparison among the populations of *M. hapla*, the numbers of total nematodes recovered per unit root weight at 1 and 4 g NVS 100 cm<sup>-3</sup> were expressed as percent of the NVS control. Percentage data were degree-arcsine( $x^{0.5}$ )-transformed before being subjected to statistical analysis. Data from Experiments 1 and 2 were first statistically compared. Other than gall indices, data from both experiments were similar. Hence, nematode population density data from the two experiments were combined. Standardized data were analysed using PROC MIXED with the contrast statement for orthogonal comparisons (SAS Institute, Cary, NC, 2000; Steel and Torrie, 1980). When no interaction between NVS and populations of *M. hapla* occurred, differences across treatment means were compared. When interaction occurred, differences among populations within a level of NVS were compared. In order to test for any correlations between the nematodes' pathogenicity and their living environments, the numbers of females per gram of fresh root in the zero NVS treatments were subjected to regression analysis against soil pH from where the nematodes were collected. Soil pH was selected as the likely factor to indicate consistent changes.

### 3. Results

#### 3.1. Description of field conditions

The four fields were within a circle of approximately 20 km diameter. Fields 1, 2, and 3 were planted with *Hosta halcyon* cv ‘Halcyon’, *Ajuga pyramidalis* cv ‘Metallicacripa’ and *Hosta franccee* cv ‘Francee’, respectively, and Field 4 was celery (*Apium graveolens*) (Table 1). All nursery fields have been under intensive fumigant and non-fumigant nematicide use for at least 20 years; whereas, only non-fumigant nematicides had been used in Field 4 for approximately 10 years. All four fields have been treated with other pesticides, fungicides, herbicides, and fertilizer per standard extension recommendations.

Soils in Fields 2 and 3 were classified as sandy, loamy sand in Field 1 and muck in Field 4 (Table 1). Ranging from 6.30 to 7.43, soil pH was significantly different among the four fields. While the fields had significant variabilities in macronutrients (data not shown), the most striking difference was that the muck soil had more than four times nitrogen than the nursery soils (Table 1).

The loamy sand (Field 1) and muck (Field 4) soils contained representatives from all major soil nematode trophic groups, including: fungivores (*Aphelenchus*, *Aphelenchoides*, and *Filenchus*), bacterivores (*Cephalobus*, *Chiloplacus*, *Eucephalobus*, *Panagralaimus*, *Plectus*, *Diploscaptor*, *Mesorhabditis*, *Protorhabditis*, and *Monhystera*), herbivores (*Criconemella* and *Paratylenchus*), and omnivore/predators (*Aporcelaimellus* and *Eudorylaimus*). Most genera appeared in both fields, but the Mononchidae (predaceous) and *Criconemella* (ectoparasites) were found only in the loamy sand (Field 1) while *Paratylenchus* (ectoparasites) were found only in the muck soil (Field 4). Nematode communities from loamy sand (Field 1) and from muck (Field 4) soil had similar richness and diversity, but differed in their ecological maturity index,  $F/(F+B)$ , and  $(F+B)/H$  indices (Table 2). Nematodes from the loamy sand soil were more ecologically mature than from the muck soil generally because of more omnivore/predators and fewer enrichment bacterivores. Additionally, the loamy sand soil had more fungivores relative to bacterivores than the muck soil, and the former had fewer fungivores and bacterivores relative to herbivores than did the latter.

Almost all of the nursery and celery fields were positive for *M. hapla*; whereas, the carrot, potato and cornfields were not (not shown). No parasitism of populations of *M. hapla* by *Hirsutella* or *Pasteuria* was observed in any of the fields (data not shown).

#### 3.2. *M. hapla* populations’ reproductive potential and response to NVS treatment

Gall indices ranged from 1.33 to 2.89 in Experiments 1 and 2 (data not shown). In Experiment 1, the glasshouse population caused more galling than the populations from

Table 2

Ecological indices analysis of Fields 1 and 4 as measured by genera richness (GR), Hill’s diversity ( $N1$ ), ecological maturity index ( $\Sigma MI$ ), ratio of fungivores to bacterivores ( $F/(F+B)$ ), and Wasilewska’s index ( $(F+B)/H$ ) of the nematode community structure

Fields	Ecological indices				
	GR	$N1$	$\Sigma MI$	$F/(F+B)^a$	$(F+B)/H^b$
1	16.00	5.89	2.47	0.23	0.80
4	13.33	7.11	1.73	0.07	29.10
SE	1.090	0.638	0.105*	0.050*	12.029*

Means and standard errors represent six replicates per field.

<sup>a</sup>Data are reported non-transformed, but were arc-sine of square root transformed prior to analysis.

<sup>b</sup>Data are reported non-transformed, but were log-transformed prior to analysis.

\* $P < 0.05$  with Tukey’s adjustment for comparisons. See methods.

Fields 2 (sand) and 4 (muck). There was no difference in gall index in Experiment 2. NVS treatment did not affect galling in Experiment 1, but the controls had more galls than the NVS treatments and there was significant interaction between NVS and populations of *M. hapla* in Experiment 2. In Experiment 3, galling ranged from 1.44 to 4.00 (not shown). Galling in the celery population (Field 4) was the lowest ( $P \leq 0.05$ ) followed by those from Fields 1 and 2. The 4 g NVS  $100 \text{ cm}^{-3}$  of soil significantly decreased galling ( $P \leq 0.05$ ).

Because there were no significant differences in the numbers of nematodes recovered from roots and no interactions between NVS and populations of *M. hapla*, data from Experiments 1 and 2 were combined. Only 13 out of the 90 nematode-infected experimental units had less than five J2 of second generation in the roots. Since these values were low and no trend due to NVS treatment was evident, they were excluded from the analysis and only females are presented (Table 3). The data show that the effect of NVS on the populations of *M. hapla* significantly ( $P \leq 0.05$ ) increased with dose. The population of *M. hapla* from Field 3 was significantly more pathogenic than the population from muck (Field 4). In Experiment 3, about 30–40% of the nematodes were J2 in all nematode treatments. The 4 g NVS  $100 \text{ cm}^{-3}$  of soil significantly ( $P \leq 0.05$ ) decreased population densities of all populations of *M. hapla* compared with the controls (Table 3). In the no-NVS controls, the population from muck soil (Field 4) had the lowest reproductive potential ( $P \leq 0.05$ ), those from loamy sand and sand (Fields 1 and 2) intermediate, and those from sand (Field 3) and the glasshouse the highest (Table 3).

Compared with the no-NVS controls, the percentage reduction in reproductive potential was similar among the populations of *M. hapla* at each NVS dose (Table 4). Across all populations, the high NVS treatment was significantly better than the low dose in reducing population densities of populations of *M. hapla* (Table 4). The

Table 3

The effects of either 0, 1, or 4 g NVS 100 cm<sup>-3</sup> of soil and population of *Meloidogyne hapla* from Fields 1–4 and glasshouse (5) on the numbers of females recovered from roots at 28 days after inoculation with 3000 eggs 500 cm<sup>-3</sup> (Experiments 1 and 2) and 300 cm<sup>-3</sup> (Experiment 3) of soil

<i>Meloidogyne hapla</i>	Experiments 1 and 2 (g NVS 100 cm <sup>-3</sup> soil*)				Experiment 3 (g NVS 100 cm <sup>-3</sup> soil†)		
	0	1	4	Mean	0	4	Mean
1	287.3	223.1 <sup>1</sup>	124.6	211.7ab	153.0bA	39.6aB	96.3
2	296.2	164.8	90.1	183.7ab	166.1bA	37.5aB	101.8
3	430.3	228.6	128.2	262.4a	253.5aA	68.9aB	161.2
4	262.4	114.8	78.3	151.8b	66.4cA	23.3aB	44.9
5	257.4	203.9	69.1	176.8ab	245.6aA	44.1aB	144.8
Mean	306.7A	187.0B	98.1C		176.9	42.7	

<sup>1</sup>Means followed by the same letters within a column or line are not statistically different ( $P \leq 0.05$ ). Lower case letters are comparisons within a column and upper case across columns.

\*Data are means of six (three per experiment) replications.

†Data are means of four replications.

Table 4

Percent reduction of the four field (1–4) and one (5) glasshouse (control) population of *Meloidogyne hapla* due to either 1 or 4 g N-Viro Soil (NVS) 100 cm<sup>-3</sup> soil treatment relative to the unamended control

<i>Meloidogyne hapla</i>	Experiments 1 and 2 (g NVS 100 cm <sup>-3</sup> soil)		Experiment 3 (g NVS 100 cm <sup>-3</sup> soil)
	1	4	4
1	40.9*	49.0 <sup>1</sup>	59.6 <sup>†</sup>
2	39.6	57.0	62.0
3	43.1	57.1	58.8
4	48.9	57.0	54.3
5	48.9	59.5	65.2
Mean	44.3B	55.9A	60.0

<sup>1</sup>Means followed by the same letters within a column or line are not statistically different ( $P \leq 0.05$ ). The two different letters following the means of NVS treatments indicate significant difference ( $P \leq 0.05$ ).

\*Data are means of six replications.

†Data are means of four replications.

percent reduction by the 4 g NVS 100 cm<sup>-3</sup> of soil in Experiment 3 was similar to that of Experiments 1 and 2.

There was an inverse relationship between soil pH of where the nematodes came from and the numbers of females per gram root in Experiments 1 and 2 ( $P = 0.029$ ) and in Experiment 3 ( $P = 0.002$ ) (Table 5). The pathogenicity of populations of *M. hapla* was not significantly affected by the interaction of soil pH and NVS in Experiments 1 and 2 ( $P = 0.23$ ) or Experiment 3 ( $P = 0.14$ ).

#### 4. Discussion

The study shows some difference in reproductive potential among the populations of *M. hapla*; hence in pathogenicity and threshold levels for management decision making. Although the populations did not differ significantly in their response to NVS treatment, the level

Table 5

Correlations between the numbers of adult females recovered per gram fresh root weight across population of *Meloidogyne hapla* from fields 1–4 at 28 days after inoculation with 3000 eggs 500 cm<sup>-3</sup> (Experiments 1 and 2) and 300 cm<sup>-3</sup> (Experiment 3) of soil and soil pHs of the locations from where the nematodes were isolated

Experiments	Df	Regression equation ( $Y = a + b \cdot \text{pH}$ )	R <sup>2</sup>	F	P
1 and 2	23	$-464.25 + (\text{pH} \cdot 114.18)$	0.20	5.43	0.029
3	15	$-700.03 + (\text{pH} \cdot 125.33)$	0.50	13.90	0.002

of suppression was dose-dependent and it is unknown how the nematode–NVS interactions may be on other soil types. The study sheds some light on complex and possible reasons for the differences in reproductive potential, response to NVS treatment, and the effect of nematode adaptation on their parasitic behaviour.

The approximately 500 DD accumulated during the study were enough heat units for *M. hapla* to initiate a second generation (Insera et al., 1982; Mennan et al., 2006). The presence of more second-stage juveniles in Experiment 3 than in Experiments 1 and 2 probably reflects differences in inoculum cohorts. Nonetheless, the reproductive potential and the response of the populations to NVS treatment were consistent among the experiments. In all cases, the population of *M. hapla* from the muck soil (Field 4) had the least reproductive potential.

As a crop not planted in these fields, tomato was a neutral and susceptible host to the populations of *M. hapla* from the field. It can be argued that the properties of the sandy loam experimental soil were much closer to Fields 1 (loamy sand) and 2 and 3 (sandy) than to Field 4 (muck soil), suggesting favouring the nematodes from Fields 1–3. The experimental soil, however, is generally more favourable to most nematodes than the muck soil (Norton, 1978). Hence, the results support the notion that the reproductive potential (and hence pathogenicity) of these populations of

*M. hapla* may be related to conditions of the production systems in which they resided. If differences in reproductive potential and/or pathogenicity are to be attributed to where the nematodes came from, however, there must be some differences or similarities among the fields. The obvious differences separating the fields' characteristics appear to be MBr use and soil texture, pH, and nitrogen.

It is generally known that soil physical (structure and texture) and chemical (pH and elements) properties have direct and/or indirect (plant mediated) effects on nematodes' living environment (Avendano et al., 2004; Norton, 1978; Melakeberhan, 1999). For example, coarse-texture (sandy) soil will be more favourable to nematodes than fine-textured (clay) soils (Norton, 1978). In part, coarse-textured soils have better aeration than fine-textured soils, whereas the reverse is true for moisture holding capacity. The nursery soils are in a separate cluster from the celery (muck) soil.

There are no reports on how nematodes from long-term exposure to nematicide and broad-spectrum pesticide like MBr behave. The failure to isolate *Hirsutella* and *Pasteuria* from the fields suggests its more due to occurrence than to MBr use. The loamy sand (Field 1) and sandy (Fields 2 and 3) soils have been under intensive MBr use compared with no MBr use in the muck soil (Field 4). While if and how either short- and long-term MBr use may have affected nematode reproductive potential directly or indirectly is unknown, the possibility should not be excluded because the nematodes have been thriving in the prevailing soil conditions.

Although soil pH values of the fields differed significantly, the lowest, 6.3 in the muck soil, would be considered near optimum in many production systems. Nonetheless, the significant correlation between soil pH of the fields where the nematodes came from and population densities of *M. hapla* recovered in roots suggests that soil pH may be a factor in influencing the reproductive potential of these populations. While how long the nematodes have been in the respective fields is unknown, soil pH does adversely affect nematode pathogenicity (Melakeberhan et al., 2004). The physio-chemical mechanism by which pH affects nematode pathogenicity however is unknown.

Nitrogenous compounds, product of most organic amendments including NVS, are known to adversely affect nematode pathogenicity (McSorley et al., 1997; Zasada, 2005; Zasada and Tenuta, 2004a, b). The significant reduction of the populations of *M. hapla* in the NVS treatments compared with the controls support this notion. In relation to nematode adaptation and reproductive potential, however, the possible role of nitrogen appears to be different from that of MBr use and soil pH. The two–three times more soil  $\text{NO}_3\text{-N}$  in the muck soil (Field 4) compared with the nursery soils (Fields 1–3) and the least reproductive potential of the population of *M. hapla* from the muck soil suggests that the nematodes may not adapt to higher nitrogen levels.

The limited ecological indices drawn from the nematode community structure analysis suggest significant differences in biological activities between the loamy sand and muck soils. While the loamy sand had more nematode genera present than muck soil, most were herbivores. High herbivore presence is one of the indicators of depleted soil conditions. The fact that the muck soil had the highest numbers of bacteriovores indicates that more decomposition happens in the rapid bacterial channel than the slow fungal channel compared with the loamy sand soil. This is consistent with the high nitrogen in the soil analysis. The inverse significant differences in the  $F/(F+B)$  and Wasilewska's index ( $F+B/(H)$ ) ratios reflect the proportion of the different feeding groups. It appears that muck, nutrient-rich soil, may be less conducive environment to herbivores than the loamy sand, indirectly confirming controlled studies on other nematodes (Melakeberhan, 1999). In addition, the muck soil had a lower maturity index, which was the result of fewer individuals from the predator/omnivore guilds. How the differences in soil ecological conditions (NCS-based) directly or indirectly relate to the reproductive potential of the populations of *M. hapla* is yet to be determined. However, nutrient availability, energy channels, and trophic structure appear to be three distinctions between the fields.

Most agricultural soils have some level of nutrient deficiency (Baligar et al., 2001) and can benefit from organic amendments that can suppress nematode population dynamics as well (Hafez and Sundararaj, 2001a, b; McSorley, 1998; McSorley et al., 1997, 1999; Riga et al., 2003; Wang et al., 2003). In addition to its soil amendment qualities (Logan and Burnham, 1995), NVS's suppression of the populations of *M. hapla* appears to be promising. However, the effect is dose-dependent for the experimental soil type, and possibly site-specific for other experimental conditions. Thus, further studies are needed to test field level of applications.

In many ways, the fields where the populations of *M. hapla* came from are representative of production systems in the vegetable and nursery industries, most affected by pesticide restrictions and in need of sustainable alternatives. It is possible that more could be achieved by obtaining a complete profile of all possible biological, physical, and chemical factors that may be directly or indirectly affecting the nematode population adaptation. Whether or not there are intra-species genetic differences and how much the differences in soil conditions contributed to the results are yet to be determined. Moreover, it is unknown how pesticide treated or untreated soil environments affect nematode parasitic behaviour. What is certain is that plant-parasitic nematodes continue to be a problem in the current input-intensive production systems. Generally, the difference in reproductive potential between the populations from the nursery soils and the muck soil suggests that nematode adaptation may be a factor that needs to be considered in identifying and in managing populations of *M. hapla*. Thus challenging the

one-management-approach-fits-all philosophy. Moreover, the study suggests that MBr use and soil pH and nitrogen may be among the factors influencing nematode adaptation. Further studies are needed to understand the physiochemical and ecological basis of nematode adaptation.

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