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Sensitivity of Nematode Life-History Groups to Ions and Osmotic Tensions of Nitrogenous Solutions

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Abstract: Guild designation of nematodes of similar trophic function and life-history strategy provides a basis for using nematode faunal analyses in an integrative assessment of soil food web condition. Omnivorous and predaceous nematodes, categorized at the upper end of a colonizer-persister (c-p) continuum of nematode functional guilds are generally not abundant in cropped soil. These nematodes are more sensitive to heavy metal concentrations than those in other c-p groups, but whether sensitivity to agrochemicals contributes to the observed low abundance of high c-p groups in cropped soils is less well understood. An exposure assay in solution was used to compare the sensitivity of nematodes representing various guilds obtained from field soils and from laboratory culture to several nitrogen sources. Nematodes in c-p groups 4 and 5 were more sensitive to nitrogen solutions than nematodes representing lower c-p groups. There were both osmotic and specific ion effects—the latter most evident in exposure of nematodes to $NaNO_2$ and $(NH_4)_2SO_4$. The RC_{50} (concentration resulting in nematode recovery of one half of that of distilled water) for $(NH_4)_2SO_4$ was < 0.052 M-N for c-p groups 4 and 5 compared to much greater values (0.34 to 0.81 M-N) for c-p groups 1 to 3. In non-ionic polyethylene glycol (PEG) solutions, osmotic tensions of 0.40 to 0.43 MPa reduced the recovery of exposed nematodes by half (RT_{50}; water potential of solution resulting in nematode recovery of one half of that of distilled water) for c-p groups 4 and 5 compared to > 1.93 MPa for c-p groups 1 to 3. RT_{50} values for urea solutions, also non-ionic, were greater than for PEG. *Caenorhabditis elegans* N2 (c-p 1) and *Meloidogyne javanica* (c-p 3) reared on solid medium and in hydroponic culture, respectively, were slightly more sensitive to specific ion and osmotic effects than nematodes of similar c-p groups obtained from soil. The greater sensitivity of c-p 4 and 5 nematodes to nitrogen solutions suggests that fertilizers may contribute to the low abundance of these nematodes in annual cropping systems. This study supports the use of nematode faunal analyses as indicators of chemical stress in soil.

Key words: c-p grouping, nematode faunal analysis, nitrogen, osmotic tension, sensitivity.

Sustainable agricultural production practices depend upon the activity of soil organisms for processes determining crop growth and quality (e.g., nutrient availability, suppression of pathogens and pests, soil tilth, etc.). Indicators of the structure and function of the soil food web would be useful tools for monitoring and understanding the effects of management practices on soil quality or health and on reclamation of damaged agroecosystems (Rapport et al., 1997). Nematodes are good bioindicator organisms because they provide an integrative assessment of soil abiotic conditions and biotic function (Bongers and Bongers, 1998; Bongers and Ferris, 1999; Freckman and Ettema, 1993).

The value of nematodes as bioindicators is rooted in the development of the maturity index (MI) proposed by Bongers (1990). Each family is assigned a colonizer-persister (c-p) value based on life-history traits. Familes assigned lower c-p values are generally characterized by high colonization ability, a short generation time, large population fluctuations, survival stages that bridge unfavorable conditions, large gonads, and production of many small eggs. Taxa assigned higher c-p values are generally less fecund, longer-lived, slower-growing nematodes. Nematodes in the lower c-p categories, able to survive adverse conditions and (or) respond to resource enrichment, are predominantly in the Secernentea, a monophyletic class. Those in the higher c-p categories are almost exclusively members of the Adenophorea, which appears to be polyphyletic (De Ley and Blaxter, 2002; Maggenti, 1981).

Recent developments in nematode faunal analysis include the characterization of two trajectories descriptive of soil food web conditions. The enrichment trajectory is based on the weighted abundance of opportunistic bacterivorous c-p 1 nematodes species (primarily secernentean families) that respond rapidly to new resources. The structure trajectory is the weighted abundance of larger, slower-reproducing bacterivore, fungivore, omnivore, and carnivore c-p 3 to 5 species (primarily Adenophorea) (Ferris et al., 2001a, 2001b).

Omnivorous and predaceous nematodes of c-p groups 4 and 5 are particularly sensitive to perturbations, here called stressors. Chemical stressors that have been observed to reduce their abundance include heavy metal contamination (Georgieva et al., 2002; Korthals et al., 1996, 2000; Parmelee et al., 1997; Zullini and Peretti, 1986); acidification (Dmowska, 1993; Ruess et al., 1996); fertilization of pastures, prairie, or forests (Kimpinski and Welch, 1971; Sarathchandra et al., 2001; Schnurer et al., 1986; Sohlenius, 1990; Sohlenius and Bostrom, 1986; Sohlenius and Wasilewska, 1984; Yeates and King, 1997); and nematicide treatment (Smolik, 1983). The diversity and abundance of c-p 4 and 5 nematodes are often limited in soils that have been subjected to conventional agricultural practices. We sampled numerous soils representing a range in

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intensity of management and found those with limited intervention (pasture or orchards with perennial cover) to have an abundance of structure trajectory taxa. In a survey of Oregon, for example, Jensen and Mulvey (1968) found that predaceous Mononchida were essentially absent from agricultural fields but abundant in surrounding undisturbed ditch banks and hedgerows. In a comparison of the nematode faunae of soils subjected to conventional, organic, and low-input farming practices, Ferris et al. (1996) found greater abundance of omnivorous Dorylaimida in the latter two systems than the former, but meager in all when compared with natural systems.

Experimental evidence is lacking for a greater sensitivity of high c-p nematode groups to chemical stressors. In a notable exception, the direct toxicity of $CuSO_4$ was measured for a range of nematodes representing both Adenophorea and Secernentea and a range of life-history groups and feeding types (Bongers et al., 2001). The authors concluded that sensitivity to $CuSO_4$ increased with c-p class. Further, of nematodes within c-p groups 4 and 5, predaceous nematodes were more sensitive than omnivores. In a solution-exposure study, nematodes of c-p groups 4 and 5 were generally more sensitive to pentachlorophenol than those with lower c-p values (Kammenga et al., 1994).

The aim of this study was to determine experimentally whether the sensitivity of nematodes to nitrogen fertilizers, a group of chemical stressors common in agricultural systems, is related to the life-history strategies of the nematode fauna. A solution-exposure assay was used to determine the direct sensitivity to nitrogen sources of nematodes obtained from several soils and from laboratory cultures. The nematodes from laboratory cultures (*Caenorhabditis elegans* and *Meloidogyne javanica*) are species that have been studied extensively. Our intent was to provide standards for comparison of responses of nematodes that have been little studied to those well characterized physiologically and ecologically. Treatments also included a non-nitrogen salt (NaCl) and non-ionic compound (polyethylene glycol) to allow assessment of the respective influence of specific ions and osmotic tension of the test solutions.

Materials and Methods

Collection of nematodes from soil: Soil samples were collected from three sites and designated as grassed (primarily a California fescue (*Festuca californica* Vasey) and clover (*Trifolium* sp.) mixture under live oak (*Quercus* sp.) located at the University of California Angelo-Margerie Coast Range Reserve (Mendocino County, CA)), riparian (under Bermuda grass (*Cynodon dactylon* L.)), and cropped (under annual crop production with a history of organic management) (Clark et al., 1998). The latter two soils were collected on the grounds of the University of California, Davis campus. None of the sites had a recent history of fertilization or application of agrochemicals. The grassed soil was an acidic sandy loam, the riparian soil a basic loam, and the cropped soil a slightly basic loam (Table 1). These soils were chosen because they contained broad ranges of nematodes of different trophic levels and life-history classifications. Soil was collected in the late spring, April 2002. Sampling in summer, fall, and winter failed to provide a similar broad range of nematode trophic levels and life-history classifications. Particularly, the abundance of omnivorous and predaceous nematodes was greatest in late spring. Samples were collected from the 2 to 12-cm depth interval at five locations (each 0.25 × 0.10 m) within each site (0.05, 0.13, and 0.15 ha for riparian, cropped, grassed soils, respectively). The samples from a site were combined, passed through a 5-mm mesh screen, and stored at 5 °C. Initial attempts to collect nematodes for exposure assays by using Cobb's sieving followed by Baermann funnel separation (48 hours) resulted in frequent infection by zoosporic fungi near the amphids of dorylaimid nematodes as observed pre-

Table 1. Characteristics of the soil from which assay nematodes were obtained.

Soil[a]	pH[b]	Osmotic potential[c] at sampling dry		Conductivity[d]	OM[e]	Texture[f]	Location	Management
Grassed	5.7	–0.01	–0.06	162	6.57	Sandy loam	UC Angelo-Margerie Reserve, Mendocino Co.	Occasional irrigation
Riparian	8.0	–0.02	–0.10	507	2.62	Sandy loam	Arboretum, UC Davis, Yolo Co.	Occasional flooding during winter
Cropped	7.4	–0.06	–0.15	234	1.47	Loam	Agronomy Field Plots, UC Davis, Yolo Co.	Sustainable Agriculture Food Systems organic plots, legumes as nitrogen source, cultivation, flood-furrow irrigation

[a] Sampled from 5 locations (each 2–12 cm deep). Samples passed through a 5-mm mesh, and then mixed together. Values are the average of duplicate analyses.
[b] pH of the supernatant of a 1:1 (soil:water) slurry.
[c] Soil osmotic potential (MPa) at moisture content at time of sampling (as is) and estimated at wilting-point (dry) moisture content (6% for Grassed and Riparian, 10% moisture content for Cropped soil). Calculated from the osmolality (Osm kg^{-1}) of the supernatant of a 1:1 (soil:water) slurry determined using a vapor pressure osmometer (Westcor, Inc., Logan, UT) (Jensen, 1982).
[d] Electrical conductivity (μS) of the supernatant of a 1:1 (soil:water) slurry.
[e] Organic matter content determined using the Walkley-Black method.
[f] Soil texture determined from particle size analysis of sand, silt, and clay in soil suspensions using the hydrometer method.

viously by Jaffee (1986) and Jaffee and Shaffer (1987). Accordingly, we used a nematode collection protocol that minimized the time from extraction to assay. Nematodes were extracted in the afternoon from 50, 200-cm^3 aliquots of each soil by Cobb's sieving and decanting followed by sugar-flotation (Ingham, 1994). Each extract was placed overnight on a modified Baermann funnel apparatus (Ingham, 1994) to separate live nematodes from debris and soil particles. The next morning, live nematodes from the funnels were placed in aerated tap water at 5 °C. The nematodes were used in an exposure assay that afternoon. Nematode communities from each soil where described using the maturity (Bongers 1990), enrichment (EI), and structure (SI) indices (Ferris et al., 2001a).

Collection of Meloidogyne javanica and Caenorhabditis elegans from culture: A hydroponic culture system, with tomato (*Lycopersicon esculentum* Mill. cv UC82) as a host, was used to produce juveniles of the c-p 3 nematode, *Meloidogyne javanica* Chitwood isolate VW4 (Lambert et al., 1992). The nematode suspension collected from the culture was placed overnight on a modified Baermann funnel apparatus to separate live juveniles from root debris. The juveniles were placed in aerated tap water at 5 °C to be used in an exposure assay the same afternoon. Juveniles of the c-p 1 nematode, *Caenorhabditis elegans* Dougherty (N2 strain), were produced in 5-cm-diam. plastic petri dishes containing rich nematode growth medium (RNGM), which is similar to standard nematode growth medium (NGM) (Brenner, 1974) but has 3-fold the amount of bacto-peptone to improve growth of bacteria on which the nematodes are feeding. Gravid adults (15) were placed onto medium seeded the previous day with *Escherichia coli* OP50. Each plate was incubated at room temperature in the dark for 6 days—enough time for the progeny of the gravid adults placed on the plate to produce juveniles. Juveniles of uniform growth stage were obtained in washings of the plate with M9 buffer (Brenner, 1974) that passed through a 500-mesh screen (25-μm pore size). This method effectively separated juveniles from adults and free eggs.

Experimental design and nematode analysis: The sensitivity of nematodes to increasing concentrations of several nitrogen sources was examined in two sets of experiments—the first set using multi-taxa suspensions of nematodes collected from each of the three soils and the second using nematodes collected from cultures of *M. javanica* and *C. elegans*. From each soil and culture system, a suspension of nematodes (500, 800, 600, 1,000, and 1,000 in grassed, riparian, cropped, *M. javanica,* and *C. elegans,* respectively) in 0.5 ml distilled water was added to a concentration range in 0.5 ml of NH_2COONH_2, $(NH_4)_2SO_4$, $NaNO_2$, and $Ca(NO_3)_2 \cdot 4H_2O$ in 1.5-ml polyethylene microcentrifuge tubes. The effect of ions on nematodes was examined using solutions of NaCl (0, 0.01, 0.05, 0.5, and 1 M) and that of osmotic tension without effect of ions by including polyethylene glycol 8000 (MW > 8000, 0, 0.26, 0.38, 0.88, 1.27 g PEG ml^{-1}). Concentrations represent those after addition of nematodes. Each treatment had two replicates, and each experiment was performed twice.

After incubation in the assay solutions in the dark for 1 day at 24 °C, nematodes were rinsed once in tap water by centrifugation (1300× *g* 1 minute for inorganic salts and 3800× *g* 2 minutes for the more viscous PEG solutions) and decanting. They were then transferred, by repeated washing of microcentrifuge tubes with tap water, into PVC tubes (21-mm i.d. × 14-mm h) covered at the bottom end with a double layer of Kimwipe tissue (Kimberly-Clark, Roswell, GA). The PVC tubes were placed on a plastic-coated paper clip in a counting dish (53-mm i.d.) in 16 ml of tap water. The paper clip prevented direct contact of tissue with the dish base and facilitated unimpeded movement of nematodes into the dish. The dishes were covered to prevent excessive water loss and, after 1 day, the exterior of tubes and paper clips was rinsed into dishes. This method separated nematodes that survived the exposure from those that did not. The nematodes in each dish were counted, and 100 were identified to genus (Bongers, 1994) and allocated to colonizer-persister groups (c-p) following Bongers and Bongers (1998). Glassware, not plasticware, was used to transfer nematodes. We found that large-bodied nematodes in the families Tripylidae, Dorylaimidae, and Mononchidae adhered to plastic surfaces, resulting in a systematic low recovery of these nematodes in all treatments (Tenuta and Ferris, unpubl. data).

Data analysis: Immediately following exposure to the 1 M $NaNO_2$-N treatments, all test nematodes appeared non-motile under a dissecting microscope. Infrequently, small nematodes of several genera were recovered from these treatments (0 to 4% of total nematodes compared to control) but were also non-motile and had disrupted body contents. They were considered to have been killed by the treatment but to have passed through the tissue because of their small size. Thus, the 1-M $NaNO_2$-N treatment was considered a positive control and the number of nematodes in those dishes was used as an estimate of non-motile nematodes passing through the tissue. That number was subtracted from all other treatments to provide adjusted estimates of surviving nematodes. Counts for c-p groups were obtained from counts of genera. Counts for each of the treatments were standardized to percentage of the control (distilled water).

We expected the survival of nematodes to be affected by specific ion effects and osmotic tension of test solutions. We attempted to separate osmotic and specific ion effects on nematode survival by determining the survival of nematodes to the osmotic tension of solutions. Thus, reduced survival in a solute compared to the non-ionic test compounds, PEG or urea, indicated a specific ion effect.

The osmolality (Osm kg^{-1}) of test solutions was de-

termined using a vapor pressure osmometer (Westcor, Inc., Logan, UT). From osmolality, the osmotic tension (MPa) calculated from the product of R (0.00831 kg MPa mol^{-1} K^{-1}) × T (°K) × osmolality (Osm kg^{-1}). A model (eq. 1), developed by Bongers et al. (2001) to describe nematode response to $CuSO_4$ concentrations, was used to analyze the response of standardized counts of nematodes to osmotic tension of solutions:

$$n_x = [n_o + s_o(1\text{-}e^{-(x/c_s)e_s})][e^{-(x/c_i)e_i}] \quad (1)$$

where, n_x = nematode number as dependent on the toxicant concentration, n_o = nematode number in control (distilled water), s_o = reference stimulation, x = concentration of toxicant (here osmotic tension), c_i = inhibition constant, c_s = stimulation constant, e_s = stimulation experiment, and e_i = inhibition exponent.

For each replicate in each experiment, the model was fitted numerically to standardized nematode counts in relation to osmotic tensions of test solutions by minimizing square error. The osmotic tension at which apparent mortality was approximately 50% of distilled water (RT_{50}, recovery tension, the osmotic tension at which n_x was 50% of n_o) was determined by numerical search using the MS Excel Solver tool (Microsoft, Redmond, WA) with the following options: automatic scaling, quadratic prediction, central differences, and Newton method. All results of numerical approximations were checked critically by visual comparison of raw data with model curves, and there was reasonable goodness of fit between observed values and values predicted using the model ($r^2 > 0.9$). RT_{50} values calculated as being outside the osmotic range of the test solutions were considered unreliable and recorded as exceeding the greatest or least value of the osmotic tensions tested. Where RT_{50} estimates for the replicate data were within test ranges, one-way ANOVA was used to test for differences between solutions or c-p groups and two-way ANOVA for differences between RT_{50} of c-p 2 nematodes between soils and test solutions using Fisher's Protected Least Significant Difference Method ($p < 0.05$). The statistical computer software, SigmaStat for Windows (Version 2.03), was used for all statistical analyses (SPSS, Chicago, IL).

Results and Discussion

A total of 71 taxa identified to genus or, in a few cases for nematodes of low abundance, to family level were obtained from the three soils. The taxa included the range of soil nematode feeding types (plant-parasites, fungal-feeders, bacterial-feeders, omnivores, predators) and c-p groups, so they were representative of the major functional guilds (Bongers and Bongers, 1998). The abundance of adenophorean taxa and higher c-p groupings increased in the order cropped, riparian, and grassed soil (Table 2). This resulted in similar order of maturity and structure index values for those soils (Table 2). Enrichment index (Ferris et al., 2001b) values were moderate for grassed and cropped soil but were higher in the riparian soil due to an abundance of the c-p 1 nematode, *Mesorhabditis*.

Like Bongers et al. (2001) we took an approach of comparing simultaneously the sensitivity of numerous taxa of nematodes to a chemical stressor. The advantage of this approach is that a range of c-p group nematodes can be examined at once. Further, nematodes of high c-p groupings (particularly 4 and 5) are difficult to rear in media or soil culture in the laboratory. However, the taxa examined were dependent upon their abundance in soil. We tried to obtain several nematode taxa for each c-p grouping by obtaining nematodes from three soils. Nevertheless, that only 5 and 4 taxa comprised c-p groups 1 and 5 does bias our results to the response of the dominant taxa in each of those groups—*Mesorhabditis* and *Aporcelaimus* for c-p groups 1 and 5, respectively.

Response to increasing concentrations of test solutions: Nematodes obtained from all soils, grouped by life history (c-p 1 to 5), were affected by increasing concentrations of nitrogen solutions (Fig. 1). The lowest concentrations of test solutions (0.05 and 0.1 M-N), except $NaNO_2$, increased the recovery of c-p 1 to 3 nematodes above that of distilled water controls. Increasing concentrations of the test solutions, except urea, reduced the recovery of all c-p groups. Survival was least in $NaNO_2$ where there was < 5% recovery for all c-p groups at the 0.5 M-N concentration.

Reduction in recovery at increasing concentrations of all test solutions was much more pronounced for c-p 4 and 5 nematodes than for lower c-p groups (Fig. 1). Generally, recovery of higher c-p nematode groups (4 and 5) increased sequentially in the order $NaNO_2$ < $(NH_4)_2SO_4$ < [$Ca(NO_3)_2$ = NaCl] < urea. Survival of c-p 4 and 5 nematodes was < 50% at the highest concentration of urea compared to distilled water treatment. PEG did not affect recovery of c-p 1 to 3 nematodes but reduced that of c-p 4 and 5 nematodes at the greatest concentrations tested. Similarly, Bilgrami and Jairajpuri (1984) found that 0.06 M solution of KNO_3 quickly incapacitated the c-p 4 nematode *Mononchus aquaticus* while the same concentration of urea, which is nonionic, did not.

The treatment effects on recovery of juveniles of *C. elegans* were greatest with increasing concentrations of $NaNO_2$; intermediate with $(NH_4)_2SO_4$, $Ca(NO_3)_2$, and NaCl; and least with urea and PEG (Fig. 2). Similar responses were evident for juveniles of *M. javanica* except that reduction of recovery was not as dramatically affected by $NaNO_2$ but was somewhat greater for urea and PEG solutions.

Osmotic tension and specific ion effects: Little is known of the selective sensitivity of nematode taxa to osmotic tension and specific ion effects of nitrogen solutions. We used the analysis of RT_{50} values for the different test

TABLE 2. Characteristics of the nematode communities obtained from soil and used in exposure assays. Listed are the genera accounting for 90% of the total nematodes obtained from each soil.

Soil	Maturity Index[a]	Enrichment Index[b]	Structure Index[b]	Genus	Class[c]	c-p Group[d]	% Abundance[e]
Grassed	3.62	34.9	92.9				
				Tylenchus	S	2	14
				Plectus	A	3	11
				Aporcelaimus	A	5	10
				Tripyla	A	3	10
				Paratylenchus	S	2	9
				Filenchus	S	2	7
				Mononchus	A	4	6
				Helicotylenchus	S	3	6
				Malenchus	S	2	6
				Mesocriconema	S	3	6
				Eudorylaimus	A	4	4
Riparian	3.13	67.4	85.5				
				Helicotylenchus	S	3	24
				Filenchus	S	2	16
				Malenchus	S	2	10
				Mesocriconema	S	3	8
				Cephalobus	S	2	8
				Tylenchus	S	2	6
				Mesorhabditis	S	1	4
				Prodorylaimus	A	4	4
				Eudorylaimus	A	4	4
				Trichodorus	A	3	2
				Prismatolaimus	A	3	2
				Ditylenchus	S	2	1
				Zeldia	S	2	1
Cropped	2.29	43.5	43.2				
				Aphelenchus	S	2	42
				Acrobeloides	S	2	19
				Chiloplacus	S	2	10
				Filenchus	S	2	7
				Tylenchus	S	2	6
				Aporcelaimus	A	5	4
				Eudorylaimus	A	4	3

[a] Determined as described by Bongers (1990).
[b] Determined as described by Ferris et al. (2001a).
[c] Taxonomic class of nematode genera: S = Secernentea and A = Adenophorea.
[d] Colonizer-persister value for nematode genera (Bongers, 1990).
[e] Proportional abundance of members of a genus to total nematodes in a soil.

solutions to distinguish the effects of osmotic tension and specific ions on nematode survival. Since urea and PEG solutions are non-ionic, their effect on the test nematodes is considered to be due to osmotic stress. The other test solutions are ionic; thus, lower survival than that in urea or PEG at the same osmotic tension may be a specific ion effect in addition to the osmotic effect.

Nematodes in c-p groups 1 to 3 were tolerant of osmotic tensions in excess of 1.93 MPa (that of the highest PEG concentration) (Table 3). c-p groups 4 and 5 were most sensitive to osmotic tension exerted by PEG, with RT_{50} values of 0.43 and 0.40 MPa estimated, respectively. Urea ameliorated the effect of osmotic tension on survival of c-p 4 and 5 nematodes; RT_{50} values were 2-fold greater for urea than PEG (Table 3). Juveniles of *C. elegans* and *M. javanica* were slightly more tolerant of osmotic tension stress relative to c-p 4 and 5 nematodes, with RT_{50} values greater than 0.90 MPa for NaCl, urea, and PEG solutions (Table 4).

Our observations on survival of nematodes of different c-p groups exposed to salt solutions are consistent with previous studies. In osmotic tension studies using nematodes of low c-p groups (1 to 3), *Rhabditis* sp. was intolerant of 0.6 M NaCl; *Pratylenchus vulnus, Hemicycliophora arenaria, Tylenchulus semipenetrans,* and *Meloidogyne hapla* intolerant of 0.8 M NaCl; and *Ditylenchus dipsaci* intolerant of 1.6 M NaCl (Viglierchio et al., 1969). Infectivity, development and egg hatch of *M. incognita* were reduced by elevated NaCl and $CaCl_2$ concentrations (Edongali et al., 1981; Edongali and Ferris, 1981). Very high concentrations of NaCl killed the c-p 1 nematode *Heterorhabditis bacteriophora* but not *Steinernema glaseri,* whereas KCl and $CaCl_2$ did not affect the survival of either nematode (Thurston et al., 1994). *Caenorhabditis elegans* was very tolerant of NaCl in solution; a 0.38-M solution only reduced survival by 13% (Khanna et al., 1997). In contrast, the c-p 4 nematode *Mononchus aquaticus* was intolerant of lower concentrations of NaCl solution within which > 0.06-M solutions

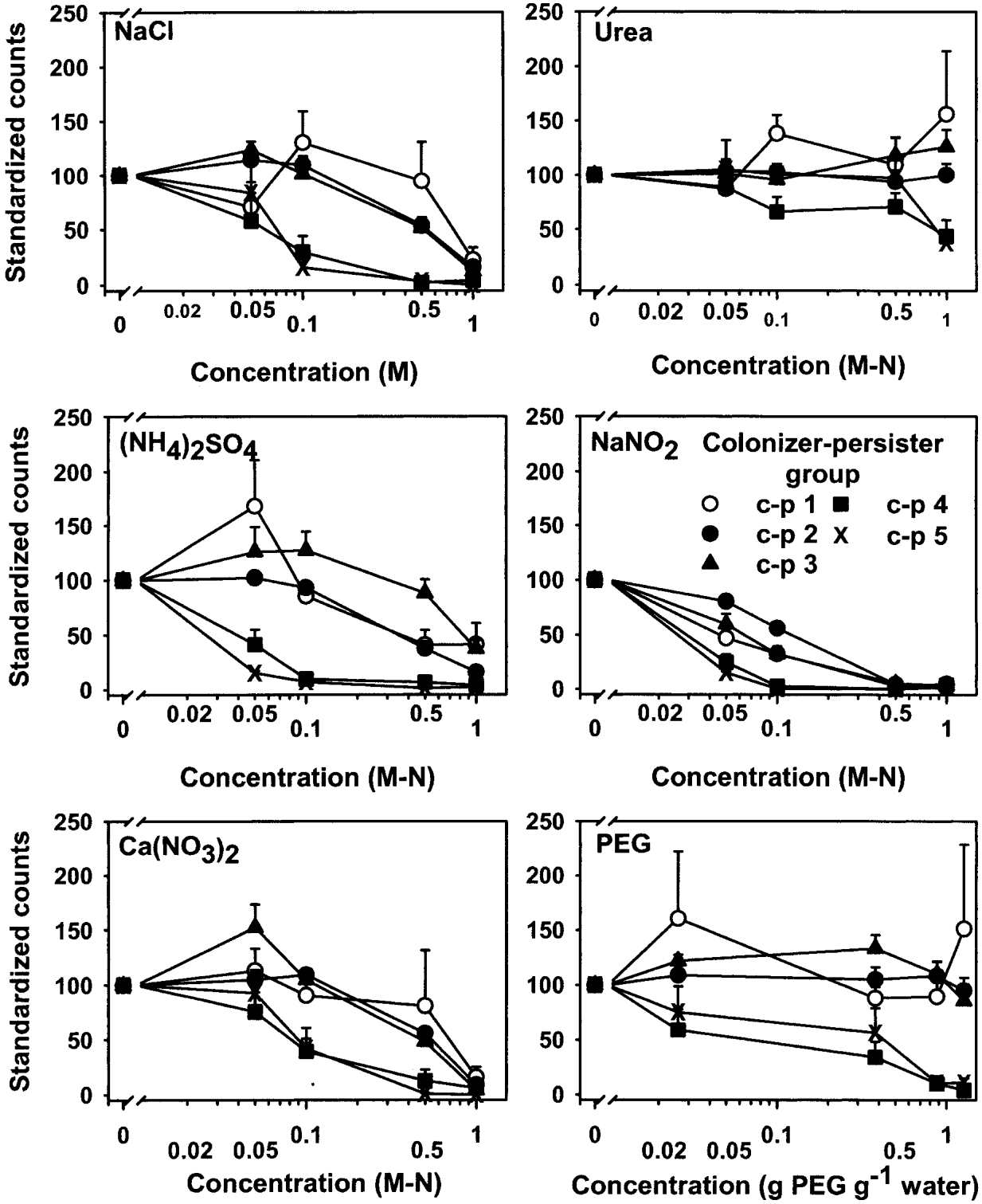

FIG. 1. Percentage of live nematodes of five colonizer-persister groups (c-p 1 to 5), recovered after exposure to six test solutions [NaCl, urea, $(NH_4)_2SO_4$, $NaNO_2$, $Ca(NO_3)_2$, polyethylene glycol (PEG)]. Nematodes were obtained from three soils (Grassed, Riparian, Cropped) and exposed to a concentration range of test solutions. Percentages are relative to the recovery for a distilled water control. Values are means of four replicates + 1 standard error of the mean. Note log-scale of x-axis for concentrations of test solutions in molarity (M) for NaCl, molarity of nitrogen in nitrogen solutions (M-N), and concentration of PEG (g PEG g^{-1} water).

incapacitated this nematode (Bilgrami and Jairajpuri, 1984).

In our study, evidence for specific ion effects of nitrogen solutions, other than urea, was provided by the RT_{50} values being generally below those for PEG and NaCl (Table 3). The similar RT_{50} values for NaCl and PEG are an indication that there was no specific effect of Na^+ or Cl^- ions. RT_{50} values for all c-p nematode

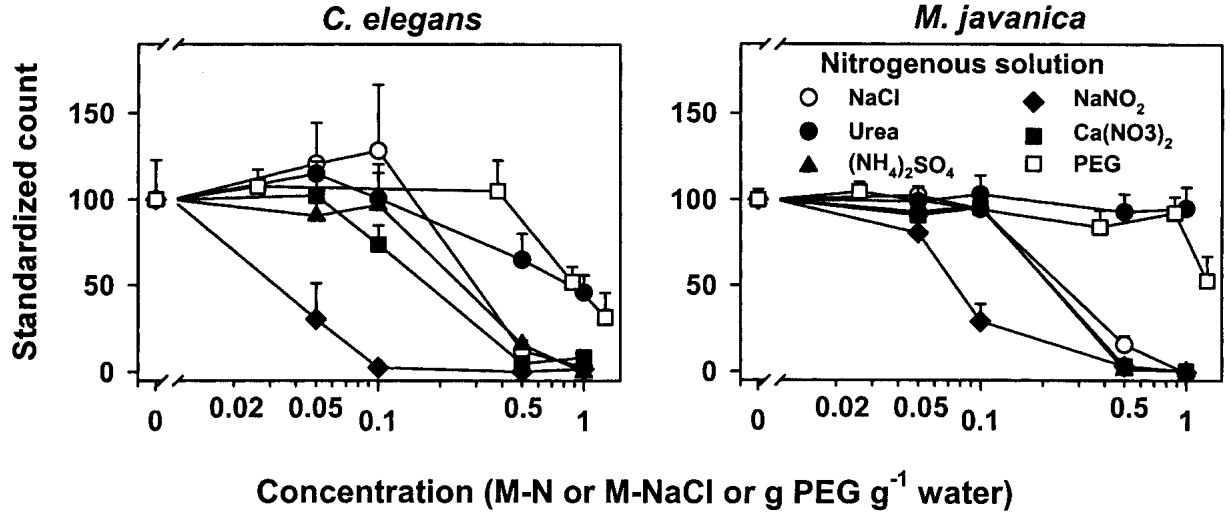

FIG. 2. Percentage of live juveniles of *Caenorhabditis elegans* and *Meloidogyne javanica* recovered after exposure to test solutions. Nematodes were obtained from laboratory cultures on agar and hydroponic solution, respectively. Percentages are relative to the recovery for a distilled water control. Values are means of four replicates + 1 standard error of the mean. Note log-scale of x-axis for concentrations of test solutions in molarity (M) for NaCl, molarity of nitrogen in nitrogen solutions (M-N), and concentration of PEG (g PEG g^{-1} water).

groups in $NaNO_2$ were very low—an indication of a lethal effect of NO_2^- ions. An NO_2^- specific ion effect on survival of *C. elegans* juveniles was evident in the lower RT_{50} value for $NaNO_2$ than for other test solutions (Table 4). Similarly, for juveniles of *M. javanica,* RT_{50} values were relatively high for NaCl, urea, and PEG solutions but lower for $NaNO_2$ and $(NH_4)_2SO_4$.

To our knowledge, the sensitivity of a broad range of species of soil nematodes to different nitrogen compounds has not previously been examined. Other researchers have examined the effect of different nitrogen compounds on survival of plant-parasitic nematodes. Generally, NO_2^- is considered the most toxic to nematodes of the nitrogen compounds present following fertilizer or organic amendment of soil (Akhtar and Malik, 2000). There is uncertainty as to the mechanism by which NO_2^- kills nematodes and other soil organisms. The most plausible mechanism is the generation of nitrous acid (HNO_2) by association with hydronium ions. Extremely low levels of HNO_2 can kill plant-parasitic nematodes (Oka et al., 1993) and other soil organisms (Tenuta and Lazarovits, 2002). Ammonium ions in soil can also kill plant-parasitic nematodes (Kaplan and Noe, 1993; Oka et al., 1993; Rodríguez-Kábana, 1986) and other soil organisms (Tenuta and Lazarovits, 2002). But again, the mechanism seems to involve generation of another compound, ammonia (NH_3) by dissociation of the NH_4^+. Nevertheless, because it is a small ion, NH_4^+ alone may be detrimental to organisms. Ammonium toxicity has been most stud-

TABLE 3. Recovery tension values (RT_{50})[a] for nematodes of different colonizer-persister groups (c-p) in test solutions.

	c-p Group					
	1	2	3	4	5	LSD[c]
NaCl	3.10[b] (0.86)	2.20 (0.28)	1.82 (0.44)	0.32 (0.07)	0.33 (0.06)	1.36
Urea	> 1.11	> 1.11	> 1.11	0.93 (0.11)	1.00 (0.09)	nd
$(NH_4)_2SO_4$	1.69 (0.51)	0.86 (0.10)	2.09 (0.19)	< 0.13	< 0.13	nd
$NaNO_2$	0.25 (0.03)	0.52 (0.03)	0.29 (0.05)	< 0.24	< 0.24	0.12
$Ca(NO_3)_2$	1.27 (0.36)	1.51 (0.08)	1.25 (0.06)	0.30 (0.06)	0.22 (0.04)	0.51
PEG	> 1.93	> 1.93	> 1.93	0.43 (0.15)	0.40 (0.20)	nd
LSD	1.63	0.48	0.74	0.32	0.35	

[a] Determined as the osmotic tension (MPa) of the test solution in which recovery of live nematodes is 50% of that in distilled water. Where nematode recovery was not reduced below 50% of that in distilled water, the RT_{50} value is indicated as being above the highest osmotic tension for that test solution.
[b] Mean and, in parentheses, ±1 standard error of the mean of four replicates.
[c] Least Significant Difference values (LSD) using one-way ANOVA for comparison of means for the main factors (c-p group or test solution) are shown ($p < 0.05$, Fisher's Protected Least Significant Difference Method). RT_{50} values set above or below the osmotic range of the test solutions were not included in the analysis. nd = no significant difference.

TABLE 4. Recovery tension values (RT_{50})[a] for juveniles of *Caenorhabditis elegans* and *Meloidogyne javanica* placed in test solutions.

	C. elegans	*M. javanica*
NaCl	0.90 (0.37)	1.28 (0.29)[b]
Urea	> 1.11	> 1.11
$(NH_4)_2SO_4$	0.89 (0.18)	0.65 (0.16)
$NaNO_2$	< 0.24	0.39 (0.05)
$Ca(NO_3)_2$	0.41 (0.08)	0.84 (0.19)
PEG	1.19 (0.30)	> 1.93
LSD[c]	nd	0.60

[a] Determined as the osmotic tension (MPa) of the test solution in which recovery of live nematodes is 50% of that in distilled water. Where nematode recovery was not reduced below 50% of that in distilled water, the RT_{50} value is indicated as being above the highest osmotic tension for that test solution.
[b] Mean and, in parentheses, ±1 standard error of the mean of four replicates.
[c] Least Significant Difference values (LSD) using one-way ANOVA for comparison of means are shown ($p < 0.05$, Fisher's Protected Least Significant Difference Method). RT_{50} values set above or below the osmotic range of the test solution were not included in the analysis. nd = no significant difference.

ied with plants where it readily enters cells resulting in depletion of energy reserves to pump the ion back into soil solution against a concentration gradient (Kronzucker et al., 2001).

Why omnivorous and predaceous nematodes seem to be more sensitive than other nematodes to specific ion and osmotic tension effects of nitrogen solutions is unknown. There are differences in cuticle structure as well as in the secretory-excretory systems of nematodes in the class Adenophorea, which are considered poorly developed, with the latter being primarily secretory in function (Wright, 1998). In contrast, the secretory-excretory systems of Secernentea (c-p 1 to 3 nematodes) are more highly developed (Bird and Bird, 1991; Wright, 1998) and have an osmoregulatory function (Nelson and Riddle, 1984). The greater sensitivity to specific ion and osmotic tension effects of higher c-p nematode groups may be due to lower osmoregulatory capabilities determining survival in test solutions or upon transfer from solutions to a more hypotonic environment of tap water.

If nematodes of different c-p groups respond similarly to exposure to gradually increasing osmotic tension, as occurs routinely in many cultivated soils, is unknown. Interestingly, dorylaimid and mononchid nematodes survive in dry litter layer of coast range forests of California (Tenuta, pers. obs.), and the dorylaim *Eudorylaimus antarcticus* survives by anhydrobiosis in soils of the Antarctic Dry Valleys (Treonis et al., 2000). Determining survival to gradually increasing osmotic tension is necessary to unambiguously conclude difference in sensitivity of nematode c-p groups to osmotic tension.

Response of c-p 2 nematodes from the soils: The recovery of nematodes in each c-p group after exposure to nitrogen solutions was determined for each of the three soils. Nematodes in the c-p 2 group were most abundant representatives in each of the three soils (Table 2). The numbers of individuals in the other groups were often few (< 30 nematodes for a group in control solutions). In that case, reliable estimation of c-p group-specific RT_{50} values for each solution was difficult as the decrease in recovery of nematodes with osmotic tension of the test solutions was variable.

The average RT_{50} value across the NaCl, $(NH_4)_2SO_4$, $NaNO_2$, and $Ca(NO_3)_2$ treatments was higher for c-p 2 nematodes from the cropped soil than from the other soils, except for $NaNO_2$ (Table 5). There was a significant interaction effect on RT_{50} value of soil and test solution. The main contributions to the interaction were the low RT_{50} for c-p 2 nematodes from the riparian soil in $(NH_4)_2SO_4$ solution and the high RT_{50} values for nematodes from the cropped soil in NaCl and Ca(NO3)$_2$ solutions (Table 5).

The differing tolerance of c-p groups of nematodes from the soils to the test solutions may be related to the conditions of the soil from which the nematodes were obtained. Nitrate rather than ammonium may be prevalent in the riparian soil due to NO_3^--rich runoff water from fertilized lawns of the UC Davis Arboretum and nitrification of NH_4^+ to NO_3^- under the moist conditions. The cropped soil receives frequent addition of animal and green manures, and increased salinity in the soil solution is associated with the animal manures and frequent irrigation (Poudel et al., 2001).

Soil concentrations of nitrogen fertilizers in relation to RT_{50} values: To determine soil conditions resulting from application of nitrogen fertilizers that are likely to impact nematode survival, RT_{50} values were converted to a parts-per-million basis. The procedure required determining the recovery concentration (RC_{50}; M-N or M NaCl) equivalent to the RT_{50} by using the strong linear relationship between concentration and osmotic tension of each test solution.

We estimate survival of c-p 4 and 5 nematodes to be compromised by soil concentrations of 149 and 111 ppm of $Ca(NO_3)_2$ and by < 74 ppm of $(NH_4)_2SO_4$. Survival of all nematode groups and juveniles of *C. elegans* and *M. javanica* would be reduced to 50% by < 170 ppm $NaNO_2$. Reduction of survival of c-p 4 and 5

TABLE 5. Recovery tension (RT_{50} as MPa) of nematodes of c-p group 2 obtained from the three soils and exposed to test solutions for 24 hours.

	Treatment						
Soil	NaCl	Urea	$(NH_4)_2SO_4$	$NaNO_2$	$Ca(NO_3)_2$	PEG	Average
RT_{50} (MPa)							
Grassed	1.47[a]	> 1.11	1.26	0.57	1.03	> 1.93	1.08 B
	(0.35)		(0.08)	(0.03)	(0.33)		
Riparian	1.91	> 1.11	0.43	0.42	1.14	> 1.93	0.97 B
	(0.21)		(0.17)	(0.08)	(0.21)		
Cropped	2.68	> 1.11	1.34	0.47	1.56	> 1.93	1.51 A
	(0.44)		(0.05)	(0.01)	(0.17)		
Average	2.02 a[b]	> 1.11	1.01 b	0.49 c	1.24 b	> 1.93	

[a] Mean and, in parentheses, ±1 standard error of the mean of four replicates. Where nematode recovery was not reduced below 50% of that in distilled water, the RT_{50} value is indicated as being above the highest osmotic tension for that test solution.

[b] Means for the main factors (soil or treatment) followed by similar letters are not different ($p < 0.05$, Fisher's Protected Least Significant Difference Method using two-way ANOVA). Analysis did not include urea and PEG treatments.

to 50% should occur at soil concentrations of 250 ppm NaCl if responses in soil are similar to that of our experimental conditions.

Concentrations of 200 and 400 ppm N, as NH_4NO_3, reduced recovery of dorylaimid nematodes (mainly the c-p 4 nematodes *Eudorylaimus* and *Dorylaimus*) in a prairie clay and sand soil, respectively, with no histories of fertilization (Kimpinski and Welch, 1971). Growth of bluegrass (*Poa* sp.) in the sand soil removed nitrogen from soil solution and resulted in better survival of the dorylaimid nematodes compared to the clay soil. Levels of nitrogen salts high enough to reduce survival of c-p 4 and 5 nematodes occur routinely with some fertilizer application techniques. Fertilizer application in bands or pockets of granules creates concentrations of nitrogen in the soil solution > 500 ppm (Eno and Blue, 1955; Nommik and Vahtras, 1982; Pang et al., 1975; Yadvinder-Singh et al., 1994). Such concentrations are above the calculated lethal levels for c-p 4 and 5 nematodes in our experiments. The extent to which localized high concentrations of fertilizer affect nematode abundance in cropped fields warrants attention.

The water potential of the soil solution in unsaturated soil is the sum of tensions exerted by dissolved compounds (osmotic) and solids (soil matrix) (Papendick and Campbell, 1980). To soil organisms, those tensions are indistinguishable (Harris, 1980). In our study, water potential of solutions was comprised solely of the osmotic tension component and assayed using PEG solution. The water potential at which most crop plants cannot obtain water is about 1.5 MPa. From our results, nematodes c-p groups 1 to 3 would be tolerant of the osmotic tension of very dry soils, whereas the same conditions would kill c-p groups 4 and 5. In this study, exposure of nematodes to osmotic tension changed rapidly as nematodes were transferred from tap water to test solutions and back. How gradual change in water potential that can occur as soil dries affects the differential sensitivity of c-p groups is uncertain.

Conclusions

An experimental basis for separating nematode taxa into functional guilds on the basis of similar response to environmental perturbation is generally lacking, although there have been some important recent studies (Bongers et al., 2001). In the context of agricultural systems, we refer to such perturbations as stressors and include agrochemicals (e.g., fertilizers, pesticides) and physical disruption of soil (e.g., tillage). This study is an advance in our understanding of the response of life-history groups of nematode taxa to an important chemical stressor in agricultural systems—inorganic nitrogen fertilizers.

C-p 4 and 5 nematodes are less tolerant of specific ion and osmotic effects of nitrogen compounds than nematodes with lower c-p values. The findings extend the results from experimental studies in which the survival of nematodes of high c-p groupings was reduced more by $CuSO_4$ (Bongers et al., 2001) and pentachlorophenol (Kammenga et al., 1994) than that of lower c-p group nematodes. Numerous observational reports complement the conclusion that the survival of nematodes of high c-p groups in soil is reduced by chemical stressors, including nitrogen fertilizers (Kimpinski and Welch, 1971; Sarathchandra et al., 2001; Schnurer et al., 1986; Sohlenius, 1990; Sohlenius and Bostrom, 1986; Sohlenius and Wasilewska, 1984; Yeates and King, 1997), heavy metals (Georgieva et al., 2002; Korthals et al., 1996, 2000); Parmelee et al., 1997; Zullini and Peretti, 1986), soil acidification (Dmowska, 1993; Ruess et al., 1996), and nematicides (Smolik, 1983). In concert, the experiments and observations support the use of c-p 4 and 5 nematodes as indicators of chemical stress in soil.

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