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Observed Differences in Life History Characteristics of Nematodes *Aphelenchus* and *Acrobeloides* upon Exposure to Copper and Benzo(a)pyrene

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Abstract. Maturity index values reflect life history characteristics often inferred by morphology. We tested the hypothesis that *Acrobeloides* and *Aphelenchus* are sensitive to chemical pollutants, opposite of what their colonizer-persister (CP) value of 2 suggests. *Acrobeloides* and *Aphelenchus* were reared at 19°C and provided diets of *Escherichia coli* and *Rhizoctonia solani*, respectively. LC_{50} values for *Aphelenchus* exposed to copper or benzo(a)pyrene (BaP) are greater than *Acrobeloides*. Copper impedes growth of *Acrobeloides* at 10 μg/g, and results in 100% mortality at 20 μg/g. In contrast, *Aphelenchus* is more resilient, with no visible impact at 20 μg/g. *Acrobeloides* and *Aphelenchus* were sensitive to much lower concentrations of BaP than copper, i.e., 0.5 μg/g inhibited development of *Acrobeloides* and 2 μg/g for *Aphelenchus*. Egg size and hatch were unaffected at 15 μg/g copper. In contrast, 0.5 μg/g BaP reduced both egg size and hatch for *Aphelenchus* but not *Acrobeloides*. Survival of *Acrobeloides* and reproduction of *Aphelenchus* responded differently to copper and BaP, implying the relationship between this classification and their sensitivity to short-term effects may be less straightforward than presumed. Refinement of index values based on empirical evidence can be used to improve sensitivity and interpretation of nematode community indices for environmental monitoring.

Keywords: environmental monitoring; fecundity; LC_{50}; maturity index; nematodes

Introduction

Nematodes have promise as bioindicators because of their ubiquity, and known response to chemical and physical perturbations to soil and water. They are under consideration in regional and national monitoring programs (Freckman, 1988; Elliott, 1994; Gupta and Yeates, 1997; Neher et al., 1998; Heinz, 2002). Although the biology and genetics of *Caenorhabditis elegans* are well known, the use of this species for ecotoxicological testing is rather artificial (Kammenga et al., 1996b). Apart from *C. elegans*, most nematode toxicity tests have been conducted using *Panagrellus redivivus*. Samoiloff (1987) used it to determine toxic effects of about 400 single chemicals. For terrestrial ecosystems, the community-based maturity index (MI) is a promising index of soil biological condition. The MI is a weighted mean of individual colonizer-persister (CP) values that have been assigned to nematode taxa into five groups (from 1 to 5) representing

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different life strategies and ecological requirements (Bongers, 1990). Smaller weights (1 and 2) are assigned to taxa exhibiting generalized "*r*-strategy" and large weights to taxa exhibiting generalized "*K*-strategy".

As a measure of the ecological successional status of a soil community, the MI may reflect the history of disturbance because succession can be interrupted at various stages by common agricultural practices, such as cultivation and applications of fertilizers and pesticides. Because nematode taxa have specific life history traits that affect their sensitivity to stress, such as reproductive period (Kammenga et al., 1997), it is thought that taxa with low CP values are assumed to be relatively tolerant or resilient to disturbance and taxa with high CP values are more sensitive to disturbance (Bongers, 1990). However, current assignments exist only at a broad level of taxonomic resolution (usually family level) and are inferred from morphology rather than based on convincing criteria. Thus, a discrepancy between the CP value of some nematode genera and their real status is inevitable (Korthals et al., 1996, 1998; Fiscus and Neher, 2002; Yeates et al., 2002).

Most previous studies of terrestrial nematode toxicity span one generation or less, and focus on exposure to heavy metals including copper, nickel, zinc (Parmelee et al., 1993; Korthals et al., 1996, 1998, 2000; Kammenga and Riksen, 1996; Bongers et al., 2001), and cadmium (Kammenga et al., 1996a, 1996b, 2000) or organics such as pentachlorophenol (Kammenga et al., 1994). These experiments were performed on bacterivores *Plectus acuminatus* and *Caenorhabditis elegans* (Kammenga et al., 1996a, 1996b, 2000). The present study is novel in observing differences in life history characteristics of two common but little studied nematode genera in response to heavy metals and polycyclic aromatic hydrocarbons (PAHs). PAHs are a major class of soil pollutants; many of them are carcinogens or mutagens in mammals and other organisms (Xu et al., 1999). Our goal was to test whether these two genera 1) respond to both classes of chemical pollutants in the same manner, and 2) have similar levels of tolerance or resilience to chemical pollution by quantifying survival, growth, and reproduction. Two studies have examined impacts of PAHs on nematode communities (Snow-Ashbrook and Erstfeld, 1998; Blakely et al., 2002) but effects of PAHs on life history characteristics of nematode genera are unavailable.

Methods

Study organisms

Two free-living parthenogenic nematodes, *Acrobeloides* sp. (Cephalobidae Filipjev, 1934) and *Aphelenchus* sp. (Aphelenchidae Fuchs, 1937), were chosen that had identical CP values of 2 (Bongers, 1990), but different diets. Each genus was isolated from a clean site with no previous significant contamination from Cu or PAH in Toledo, Ohio (Lucas County, 41°37′48″N, 83°38′50″W) *Acrobeloides* is generally considered to be a bacteriovore (Yeates et al., 1993) but at least some species may graze fungal hyphae (Ruess and Dighton, 1996). *Aphelenchus* is usually considered a fungivore (Yeates et al., 1993). A CP value of 2 suggests both genera have life history characteristics associated with tolerance or resilience to environmental disturbance. Populations of these two genera are reported to respond to environmental stress in a manner that contrasts their assigned CP value, based on morphology (Korthals et al., 1998; Fiscus and Neher, 2002). We cultured *Acrobeloides* on strain OP50 *E. coli* bacteria and *Aphelenchus* on *Rhizoctonia solani* fungi. Initial cultures of OP50 were started in liquid LB (Wood, 1988). *E. coli* were dispersed on 1:50 diluted NGM (Wood, 1988). Stock cultures of *R. solani* AG2-1 F56L were reared on Difco PDA. Experiments were performed with *R. solani* grown on CMA for improved visibility. All experiments were conducted using 60-mm diameter plastic Petri dishes.

Measurements

In these experiments, characteristics of survival, size and reproduction were measured for each nematode species exposed to each contaminant, copper and benzo(a)pyrene (BaP). Experiments included quantitative estimates of survival (LC_{50}) with acute exposure without food (Camargo et al., 1998), size with food throughout Generation 1 (size and rate), and reproduction (egg size, hatch) with food under acute (Generation 1) and more chronic (Generation 2) situations. To be applicable to environmental monitoring programs, chronic exposures are necessary to represent soils polluted for many decades. All experiments were conducted at 19 ± 0.5 °C.

Acute survival experiments

Acute survival experiments were conducted as a completely randomized design. Each genus was tested in at least four concentrations of copper and BaP. For each of three replicates of each concentration, 10 adults (J4, just entering adult stage, no eggs laid) were placed on a culture dish and incubated for 5 days after which percent mortality was quantified. Death was determined to occur if an individual did not respond when touched by a probe. Mortality in treatments was expressed as a difference from control treatments. A lethal concentration (LC_{50}) was computed for each genus and contaminant type combination.

Growth and development experiments

Growth and development of each genus were quantified on sterile media with a range of concentrations of copper and BaP. For *Acrobeloides*, 0, 5, 10, 15 and 20 μg/g copper and 0, 0.125, 0.25, 0.5 and 1 μg/g BaP were tested. For *Aphelenchus*, 0, 10, 20 and 40 μg/g copper and 0, 0.5, 1 and 2 μg/g BaP were used. Copper and BaP concentrations were prepared from stock solutions of copper chloride (4000 μg/g) and BaP dissolved in acetone (1000 μg/g). Preliminary experiments showed no statistical difference in survival and growth in media amended with or without acetone. These concentrations were chosen because neither species of nematode could survive or reproduce on copper (2,550–7,290 μg/g) or BaP (34.4–30,800 μg/g) concentrations observed in the field (Blakely et al., 2002; Neher et al., unpublished data). Adjustment to pH of 5 was achieved using 0.25 M sodium bicarbonate. Chosen concentrations were based on preliminary tests demonstrating that greater concentrations were lethal and/or impeded growth of a respective genus. Length and width of progeny were quantifed using KS-300 video imaging software (Axiovision 2.0, Carl Zeiss Vision GmbH, Hallbergmoos, Germany) at 4, 8, 12, 16, and 20 days after gravid females were removed from the plates. Software was calibrated using a stage micrometer. Inoculation consisted of placing gravid females on the plate, and removing them after laying eggs for one day. Each treatment was comprised of 15 culture dishes (3 replicates × 5 measurements through time). Biomass (ng C) was computed according to Andrássy (1956).

Reproduction experiments

Reproductive output was measured as egg size and hatch in two successive generations. Initially, 10 gravid females were placed on each culture dish. In Generation 1, the adults were removed after 1 day of laying eggs. In Generation 2, egg production of single females was monitored. Length and width measurements of at least five eggs per plate and three replicate plates per time (total of 15–20 nematodes per treatment per time) were measured using methods described for growth and development experiments. *Acrobeloides* laid eggs on the surface of agar media. For Generation 1 images, eggs were scraped from the agar and placed in a water-filled Petri dish prior to imaging, whereas they were imaged directly on the agar surface in Generation 2. *Aphelenchus* laid eggs throughout the agar matrix. Therefore, to measure egg size, it was necessary to destructively sample by melting agar chunks at 100 C, diluting it to prevent re-solidification, and collecting eggs that settled to the bottom of the Petri dish. Hatch was quantified as the number of juveniles that emerged from eggs for separate dishes with identical treatments but not sampled destructively.

Statistical analysis

Linear regression was performed with survival as a dependent variable and contaminant concentration as the independent variables. LC_{50} values were determined using a linear regression model computed with survival and contaminant concentration as dependent and independent variables, respectively. Growth and reproduction experiments were analyzed using a two-way analysis of variance with size or hatch as dependent variables and contaminant concentration and day as independent variables. Separate statistical models were computed for each genus and contaminant type combination. To meet assumptions of normality, proportion of eggs hatched was transformed as arcsine of the square root and worm weight was transformed as $\ln(x + 0.1)$. *Post-hoc* means comparisons were computed with a Bonferroni adjustment to prevent excessive Type I errors. Regression and analysis of variance were performed using GLM and MIXED procedures of SAS Version 8 (SAS Institute, 2000), respectively.

Results

Survival

Contrary to our prediction of relative tolerance, based on similar CP value assignments of 2, *Acrobeloides* and *Aphelenchus* responded differently to copper and BaP. *Acrobeloides* was more sensitive to both copper and BaP than was *Aphelenchus* (Table 1).

Development

Nematodes in culture for 24 months appeared to mature more slowly and be more sensitive to contaminants than those in culture for 14 months in non-contaminated media prior to experiments (Figs. 1, 2). *Acrobeloides* were adults at 12 days and post-reproductive at 16 days when cultured for less than 14 months prior to acute exposures.

Table 1. LC_{50s} of two nematode genera after 5 days exposure to copper and benzo(a)pyrene at 19 ± 0.5 °C

Nematodes	Copper	Benzo(a)pyrene
Acrobeloides	27.8^{ns}	3.9*
Aphelenchus	74.5**	12.3*

Expressed are lethal oncentration doses (μg/g) for 50% of the population (LC_{50}) for a range of five concentrations for *Acrobeloides* and four concentrations for *Aphelenchus* ($n = 30$ worms per concentration).
$^{ns}p > 0.05$, measured among concentrations for each genus and contaminant concentrations.
$^{*}p < 0.05$, measured among concentrations for each genus and contaminant concentrations.
$^{**}p < 0.0001$, measured among concentrations for each genus and contaminant concentrations.

Development was delayed after 24 months in culture, with *Acrobeloides* not reaching an adult stage until at least 20 days. Patterns were similar for *Aphelenchus* when cultured for 14 months, and development rates were inconsistent among individuals after 24 months of culture. Development of *Acrobeloides* was stimulated when exposed to 5 μg/g copper, but inhibited at greater than or equal to 10 μg/g of copper in early-stage cultures. Difference in response to copper concentration diminished after 24 months in culture (Fig. 1). In contrast, development of *Aphelenchus* was unaffected by copper at concentrations ranging from 5 to 20 μg/g. Development of *Acrobeloides* was impeded more by BaP than copper (Fig. 2). For example, 0.25 μg/g BaP inhibited development in 14- and 24-month cultures. Similarly, development of *Aphelenchus* was also impeded more by BaP than copper. However, *Aphelenchus* was able to tolerate greater concentrations of BaP than *Acrobeloides* before any effect was observed.

Reproduction

With acute (single generation) exposure to either copper or BaP, reproduction was inhibited at concentrations below the threshold for survival (Table 1, Figs. 3–5). Similar to survival, reproduction of *Acrobeloides* was more sensitive to acute exposure of copper or BaP than *Aphelenchus* at matching concentrations. Copper impacted the reproduction of each species differently. Egg size and hatch of *Acrobeloides* was not affected ($p > 0.5096$) at 0–15 μg/g copper (Figs. 3a, c). In contrast, hatch of *Aphelenchus* was less at 20 and 40 than 0 and 10 μg/g copper ($F = 30.32$, $p < 0.0001$, Fig. 5c). Egg size remained uniform at 0–20 μg/g copper ($p = 0.8428$, Fig. 5a). Although reproduction was impacted negatively at much smaller concentrations of BaP than copper, general species patterns were similar to those observed for copper. Specifically, acute exposure reduced egg size ($F = 5.40$, $p = 0.0028$) and hatch ($F = 4.28$, $p = 0.0029$) at 0.5 and 1 compared to 0, 0.125 and 0.25 μg/g BaP (Fig. 4a, c). Size of *Aphelenchus* eggs ($F = 4.43$, $p = 0.0153$) and hatch ($F = 14.42$, $p < 0.0001$) were reduced at 0.5, 1 and 2 compared to 0 μg/g BaP (Fig. 5b, d).

Acrobeloides was followed through Generation 2 of reproduction to determine whether longer,

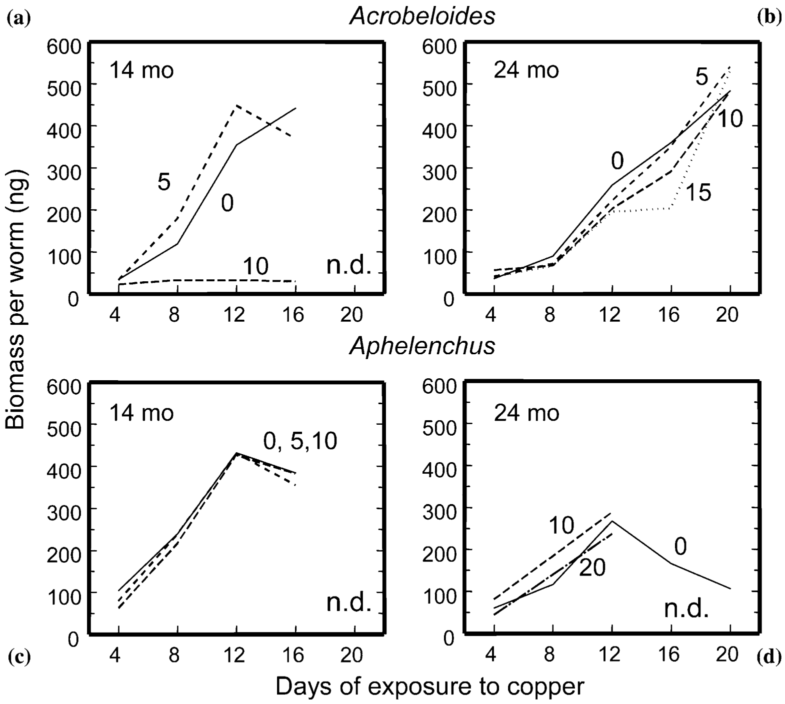

Figure 1. Effect of contrasting concentrations of copper on subsequent size *Acrobeloides* (a, b) and *Aphelenchus* (c, d) after 14 (a, c) or 24 (b, d) months of laboratory culture on 1.0% water agar. Mean biomass after 4, 8, 12, 16 or 20 days of exposure to 0 (solid), 5 (short dash), 10 (long dash), 15 (dotted), and 20 (alternating long and short dash) µg/g at 19 ± 0.5 °C. Values represent means of three replicates of 15 worms measured destructively at each time interval. Absence of a line style at a particular time indicates no survival of nematodes and, thus, biomass was not determined (n.d.).

chronic exposure impacted reproduction differently than acute, shorter exposures to copper and BaP. In Generation 2, generation time remained between 20.0 and 20.7 days for 0, 5 and 10 µg/g copper ($p = 0.0787$) and between 19.0 and 20.5 days for 0, 0.25 and 0.5 µg/g BaP ($p = 0.2500$). The proportion of eggs that hatched increased ($F = 7.26$, $p = 0.0033$) when exposed to 5 and 10 µg/g copper (Fig. 3d). When exposed to BaP, hatch was no longer affected ($p = 0.8321$, Fig. 4d). Neither copper nor BaP affected egg or juvenile size ($p > 0.08$).

Discussion

To our knowledge, this is the first report of LC_{50} values for nematodes to PAHs. Generally, *Aphelenchus* appeared more tolerant of both copper and BaP than *Acrobeloides*. In related studies, we have observed a similar pattern for other PAHs including phenanthrene, but the opposite was true for fluoranthene (Li and Neher, unpublished data). For comparison, *Clarkus*, which is assigned a CP value of 5, was more sensitive to all chemicals than *Acrobeloides* and *Aphelenchus* (Li and Neher, unpublished data). The contrasting ranks among genera for each toxicant suggest responses are compound-specific and possibly linked to different rates and routes of exposure and metabolism (Wright, 1998). Our data support Korthals et al. (1996) who suggest that genera with similar CP values can have different toxicological responses. For example, estimates of LC_{50} for copper in this study support those of Korthals et al. (1996) for *Acrobeloides and Aphelenchus*. In comparison, copper reduced survival of adult and juveniles of *Plectus acuminatus* at 10 µg/g copper with a NOEC (no observed effect concentration) of 32 µg/g (Kammenga et al., 1996b). Even with similar CP value assignments, it is perhaps not surprising that the abilities of *Acrobeloides* and *Aphelenchus* to

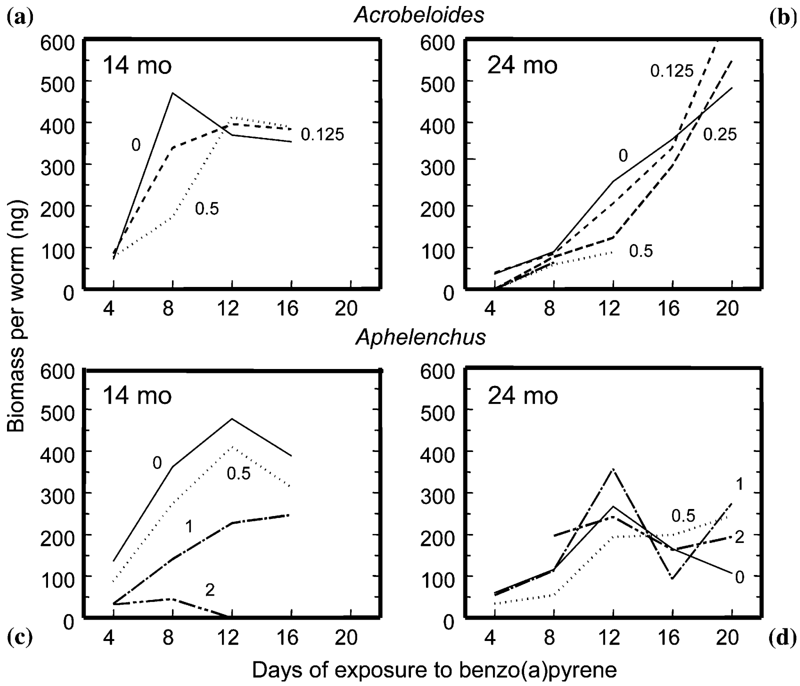

Figure 2. Effect of contrasting concentrations of benzo(a)pyrene on subsequent size of *Acrobeloides* (a, b) and *Aphelenchus* (c, d) at 14 (a, c) or 24 (b, d) months of laboratory culture. Mean biomass after 4, 8, 12, 16 or 20 days of exposure to 0 (solid), 0.125 (short dash), 0.25 (long dash), 0.5 (dotted), 1 (alternating long and short dash), and 2 (alternating one long and two short dashes) μg/g at 19 ± 0.5 °C. Values represent means of three replicates of 15 worms measured destructively at each time interval. Absence of a line style at a particular time indicates no survival of nematodes and, thus, biomass was not determined (n.d.).

survive exposure of different chemicals contrasts. Variability among taxa in tolerance to single ion types has been observed (Van Gundy, 1965).

Nematode survival strategies include large reproductive capacity by high fecundity and short generations, mechanisms to synchronize a life cycle with availability of food, and physiological triggers to indicate presence of suitable food supply (Wharton, 2002). Both *Acrobeloides* and *Aphelenchus* have high potential reproductive capacity. Although generation time of *Acrobeloides* is shorter than *Aphelenchus*, *Aphelenchus avenae* has the ability to survive at least two years in unfavorable conditions such as desiccation or osmotic stress through anhydrobiosis (Higa and Womersley, 1993; Charwat et al., 2002).

Although *Acrobeloides nanus* is capable of anhydrobiosis, longevity under these conditions is shorter than for *Aphelenchus* (Demure et al., 1979; Nicholas and Stewart, 1989; Womersley et al., 1998). It is reasonable to assume that longevity in an anhydrobiotic state can serve as a mechanism for *Aphelenchus* to survive greater concentrations of copper, BaP, and phenanthrene. The physiological trigger among nematode species may vary. For example, trehalose is known to play a role, but not the sole mechanism for surviving desiccation (Womersley and Higa, 1998). However, cuticle permeability may be a major route, in addition to food intake, for uptake of soil contaminants (Howell, 1983; van Straalen and van Gestel, 1993; Kammenga et al., 1994). Unlike *Acrobeloides*, it is well established that *Aphelenchus* has a cuticular structure and metabolism to avoid stress caused by extreme drought and temperatures (Crowe and Madin, 1975; Higa and Womersley, 1993; Womersley et al., 1998). These differences in cuticle morphology may be pre-adaptations, providing benefits for which they were not selected, and therefore appear adaptive (Rożen, 2000). CP2 organisms may have adaptive life history traits such as early maturation and high fecundity.

Development inhibition may be a much more sensitive index of toxicity than mortality of

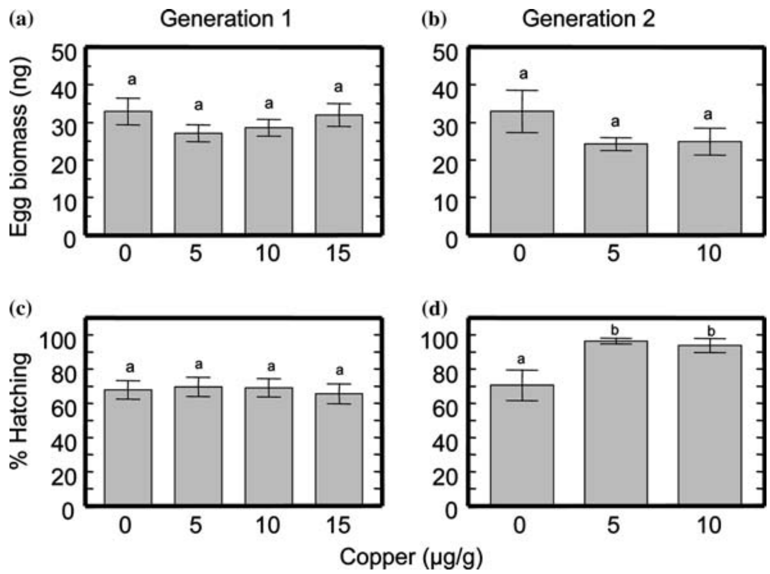

Figure 3. Effect of two generations of exposure to contrasting concentrations of copper to egg biomass (a, b) and percentage of eggs that hatched (c, d) of *Acrobeloides*. For ease in comparison, response for the first (a, c) and second (b, d) generations are illustrated. Mean ± standard errors are illustrated for 15–20 worms per treatment at each time interval. Contrasting letters indicate a significant difference at $p < 0.05$ among concentrations within a graph panel, determined by a Bonferroni means comparison. Absence of a line style at a particular time indicates no survival of nematodes and, thus, biomass was not determined (n.d.).

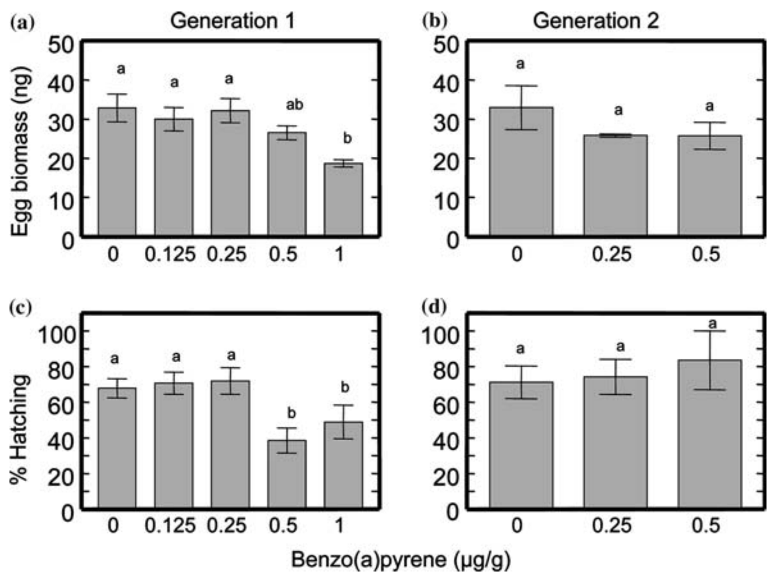

Figure 4. Effect of two generations of exposure to contrasting concentrations of benzo(a)pyrene to egg biomass (a, b) and percentage of eggs that hatched (c, d) of *Acrobeloides*. For ease in comparison, response for the first (a, c) and second (b, d) generations are illustrated. Mean ± standard errors are illustrated for 15–20 worms per treatment at each time interval. Contrasting letters indicate a significant difference at $p < 0.05$ among concentrations within a graph panel, determined by a Bonferroni means comparison.

nematodes (Samoiloff et al., 1980; Vranken and Heip, 1986; van Straalen and van Gestal, 1993). However, this may not be the case for all nematode species. For example, Haight et al. (1982) noted that effective concentrations inhibiting growth of *Panagrellus silusiae* were greater than the LC_{50}.

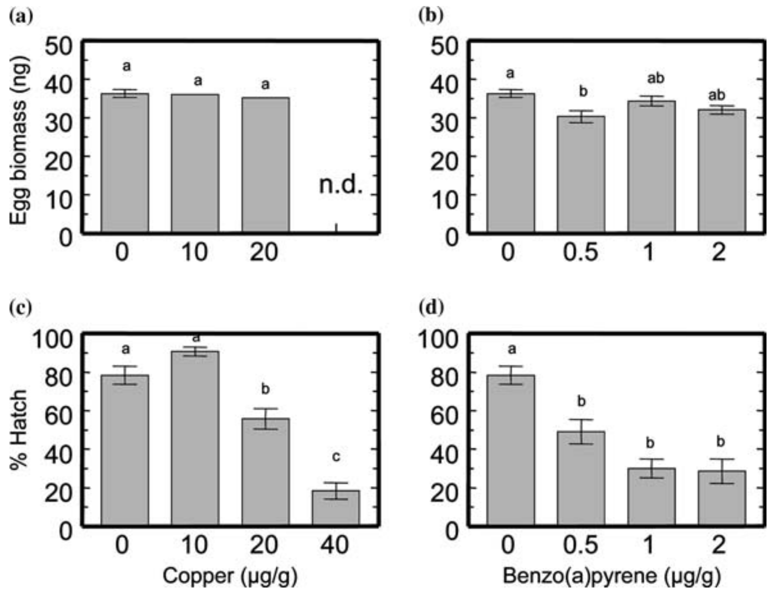

Figure 5. Effect of acute exposure of contrasting concentrations (μg/g) of copper (a, c) and benzo(a)pyrene (b, d) on egg size (c, d) and hatch (c, d) of first generation of *Aphelenchus*. Mean ± standard errors are illustrated for 15–20 worms per treatment at each time interval. Contrasting letters indicate a significant difference at $p < 0.05$ among concentrations within a graph panel, determined by a Bonferroni means comparison. Standard error bars that are not visible are smaller than the symbols. n.d. = not determined.

Vranken and associates studying mercury toxicity to *Monhystera disjuncta* noticed an 'all or none' response; some individuals developed as fast as those in the blank, whereas others died very quickly. We observed a combination of these observations to be true. Generally, development was sensitive to lower concentrations than the LC_{50}s. *Acrobeloides* was more prone to the 'all or none' response than was *Aphelenchus*.

Population growth rate is determined by a combination of fecundity and generation time, products of development rate (Vranken and Heip, 1986). In our experiments, 5 μg/g copper stimulated and 10 μg/g copper slowed development of *Acrobeloides*, but not of *Aphelenchus* in cultures less than 14 months. BaP slowed development of both *Acrobeloides* and *Aphelenchus*. Although it may be surprising initially to observe a stimulation of *Acrobeloides* development at 5 μg/g copper, similar patterns of accelerated development at low concentrations of copper have been observed for collembolans (45 μg/g) and earthworm cocoon production (60 μg/g) (Bengtsson et al., 1983; van Gestel et al., 1989). Phenanthrene retarded development of *Acrobeloides* at a rate similar to that of BaP (Li and Neher, personal observation).

Very few studies exist that report effects of either heavy metals or PAHs on development of nematodes other than *Caenorhabditis elegans* (Kammenga et al., 2000). One example is the study of a typical bacterivorous marine nematode, *Diplolaimella* spp. whose development time prolonged with increasing copper concentrations (Vranken and Heip, 1986). Development of *Diplolaimella* was delayed more by a given concentration of copper than by mercury or lead. Any toxicant that interferes with uptake and metabolism of nutrients may result in 'physiological starvation' (Popham and Webster, 1980; Postma and Davids, 1995), thus slowing population growth.

Reproduction in both *Aphelenchus* and *Acrobeloides* was more sensitive than were survival and development to copper and PAHs. Results for *Diplolaimella* were similar in pattern and concentration of copper (Vranken and Heip, 1986). In contrast, Kammenga et al. (1996a) suggest that survival and duration of juveniles (somatic growth and survival) may be more important to population growth rate than fecundity when *Plectus* *acuminatus* is exposed to cadmium. Egg size and hatch were affected adversely by lower concentrations of PAHs than copper. PAHs are more toxic than copper on *Acrobeloides* reproduction, affecting egg size and hatch. The impact of copper and BaP has a different mechanism for *Aphelenchus*. Copper reduced hatch rate but not egg size, and BaP reduced both egg size and hatch rate. Camargo et al. (1998) noted that *A. avenae* juveniles were more sensitive to mercury exposures than adults. The egg is usually the most resistant stage of the life cycle (Bird and Bird, 1991), serving as an important survival phase in the life cycle of the nematode. Once out of the egg, the nematode's first line of defense against its environment is the cuticle, which serves as a protective coat and permeability boundary, and hydrostatic-skeletal system. Nematode cuticle is comprised of three primary layers (cortical, median and basal), each which may be subdivided, reduced or variously elaborated. The epicuticle may act as a hydrophobic barrier to diffusion (Blaxter and Robertson, 1998), and has been suggested to be the major region controlling cuticular permeability (Bird and Bird, 1991). Another vulnerable period of a nematode life cycle is molting when the cuticle undergoes ultrastructural changes (Bird and Bird, 1991). Cuticle changes with each molt and each new cuticle has a distinct composition of structural proteins (Blaxter and Robertson, 1998).

As isolates in our study were maintained in culture for increasingly longer periods of time, they became more sensitive to toxicants, generation time lengthened because of delayed development, and egg hatch increased. Earlier reproduction and increased reproductive effort have been reported for soil nematode *C. elegans* (Kammenga et al., 2000), collembolans (Posthuma et al., 1992; Tranvik et al., 1993), isopods (Donker et al., 1993) and Diptera (Postma and Davids, 1995). Typically, metal-adapted invertebrates have a shorter life cycle and greater reproductive effort (Posthuma and van Straalen, 1993). Population changes may result either from changes in gene pool (adaptation) or phenotypic plasticity (acclimation) (Posthuma and van Straalen, 1993; Kammenga and Riksen, 1996; Kammenga et al., 1997, 2000, 2001). These changes suggest that presence of toxicants in the environment is a selective force. However, generalizations about toxicants in the environment acting as a selective force on soil invertebrates is impossible because of the lack of consensus on the relationship between genetic variability and exposure to toxicants (Hoffmann and Merilä, 1999; van Straalen and Hoffmann, 2000) and paucity of multi-generation effects of toxicants (Popovici and Korthals, 1995; Postma and Davids, 1995; Kammenga et al. 2000).

Life history patterns are relevant to ecotoxicological testing because population consequences of toxic action depend on the tradeoffs between life history components. Technically, tradeoffs must be measured in terms of energy allocation and/or genetics. For *Aphelenchus*, it seems that copper stress reduced hatch but not size of eggs. Perhaps this is a trade-off between reproduction and survival. In conclusion, we determined that *Acrobeloides* is more sensitive than *Aphelenchus* to copper and BaP, implying that a CP value of 2 is inaccurate. Our current knowledge is insufficient to place all nematode taxa in correct CP groups (Korthals et al., 1996). Refinement of index values based on empirical evidence can be used to improve sensitivity and interpretation of nematode community indices.

Acknowledgments

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