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Environmental Toxicology and Chemistry, Vol. 26, No. 1, pp. 73–79, 2007

Printed in the USA
0730-7268/07 $12.00 + .00

INCREASED RESPONSE TO CADMIUM AND *BACILLUS THURINGIENSIS* MAIZE TOXICITY IN THE SNAIL *HELIX ASPERSA* INFECTED BY THE NEMATODE *PHASMARHABDITIS HERMAPHRODITA*

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(*Received* 24 *February* 2006; *Accepted* 28 *June* 2006)

Abstract—To determine the effect of nematode infection on the response of snails to selected toxins, we infected *Helix aspersa* with 0-, 0.25-, 1-, or 4-fold the recommended field dose of a commercial nematode application for agricultural use. In the first experiment, the snails also were exposed to cadmium via food and soil at concentrations of 0, 30, 60, 120, or 240 mg/kg in a full-factorial design. In the second experiment, snails were infected with nematodes and also fed either Bt (expressing *Bacillus thuringiensis* toxin) maize or non-Bt maize. The snails were weighed at the beginning and end (after four weeks) of the experiments, and mortality was checked daily. Neither exposure of snails to nematodes nor exposure of snails to cadmium or Bt toxin affected the survival rates of snails. The number of dead snails was highest for combinations of nematode treatments with cadmium concentrations of 120 and 240 mg/kg. In both experiments (Bt and cadmium), the growth rate decreased with increasing nematode dose. The Bt maize was not harmful to the snails in the absence of nematodes, but infected snails grew faster when fed non-Bt maize. The growth rate of snails exposed to cadmium decreased with exposure to increasing Cd concentrations and differed significantly between the no-nematode treatment and the treatments with nematode doses of one- and fourfold the recommended field dose. Snails treated with the highest dose of nematodes accumulated the highest cadmium concentrations.

Keywords—Heavy metals Multiple stressors Nematodes Snails *Bacillus thuringiensis*

INTRODUCTION

Living organisms in their natural habitat usually are not completely healthy, so the results of ecological laboratory tests on healthy individuals may lead to erroneous conclusions regarding the effects of toxicants, underestimating their influence. A growing number of studies have investigated how multiple stressors alter the response of organisms exposed to toxic substances; the results of multistressor exposure have been found to differ vastly from those obtained during studies with single toxicants (see, e.g., [1–3]).

Terrestrial gastropods likely play an important role in nutrient cycling within terrestrial ecosystems and contribute to the transfer of nutrients and certain pollutants to higher levels of terrestrial food chains—these animals often serve as prey or host for a variety of animals [4,5]. On the other hand, some gastropods, including *Helix aspersa*, our study animal, is regarded as a pest of many crops [6]. *Helix aspersa* often is used in ecotoxicological tests and biomonitoring of metal pollution [7]. We also chose this species of snail because it is quite resistant to the parasitic nematode *Phasmarhabditis hermaphrodita* (http://www.nysaes.cornell.edu/ent/biocontrol), which was used in the present study as one of stressors. Snails can be infected with, but cannot be immediately killed by, the nematodes, as we observed in the preliminary study. Therefore, we presumed that this species is appropriate for studying the influence of multiple stressors (i.e., infection with nematodes, metal pollution, and Bt toxin).

Two of the three stressors applied in the present study of *H. aspersa* (Bt toxin Cry1Ab and parasitic nematodes) are widely used for crop protection, and the third, cadmium, is a common pollutant in metal-contaminated areas. Testing the effects of three toxicants on a single-receptor species allowed us to study different aspects of multistressor exposure. First, we were able to examine how nematode-infected snails respond to a biopesticide or a polluted environment. Second, different types of crop protection methods often are used together against several kinds of pest, and in the present study, we could observe how these treatments affect snails. Third, the present study modeled a scenario in which snails inhabiting a polluted area also are exposed to management practices for biological control.

The nematode species used as the infecting factor was *P. hermaphrodita* (Schneider), a bacteriophagous, rhabditid nematode commonly applied as a biological control for many crops. It can infect and kill a wide range of pest species (i.e., slugs) from the families Arionidae, Limacidae, and Milacidae (Mollusca: Gastropoda: Stylommatophora). The nonfeeding, third-stage (dauer) juvenile is capable of infecting susceptible species by entering the shell cavity beneath the dorsal surface of the slug mantle, then releasing the symbiotic bacteria (the most important of which is *Moraxella osloensis*). *Phasmarhabditis hermaphrodita* grows into a hermaphroditic adult and reproduces within the shell cavity [8]. Slug feeding is markedly reduced from the onset of infection (http://www.nysaes.cornell.edu/ent/biocontrol/). Following the death of the host slug, the nematodes spread out over the entire cadaver, feed, and reproduce, eventually producing dauer juveniles that move into the soil, where they can infect new hosts. *Phasmarhabditis hermaphrodita* can be cultivated on nutrient-rich

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medium, either xenically, with mixed populations of bacteria, or monoxenically, with one of a number of species of bacteria. Infective juveniles can be applied as a drench or spray to the soil to protect plants from slug damage in the field (http://www.nysaes.cornell.edu/ent/biocontrol/).

The garden snail *H. aspersa* is the most susceptible to *P. hermaphrodita* when its body weight is approximately 1 g. The infective juvenile nematodes probably invade the snails through the breathing pore underneath the mantle or through other natural openings. The course of infection in the garden snail is similar to that in slugs (http://www.nysaes.cornell.edu/ent/biocontrol/).

The most successful transgenic crops have been those that produce crystal protein endotoxin (Cry) from *Bacillus thuringiensis* (Bt), especially those that express Cry1A toxins [9]. The first Bt plant pesticide, a variety of corn producing Cry1Ab toxin, was registered with the U.S. Environmental Protection Agency in 1995 (www.epa.gov/pesticides/biopesticides/pips/bt_brad2/2-id_health.pdf). The transgenic corn was developed mainly for use against *Ostrinia nubilalis*, the European corn borer, the larvae of which feed on the corn stalk, where they are relatively safe from exposure to pesticides applied as a foliar spray [10].

For the crystal forms of Bt toxin that are produced by bacteria to kill insects, these crystals must be ingested. In the alkaline insect midgut, the crystals dissolve into protoxins, which are cleaved by proteases into active toxins. Larval ingestion of lethal Bt toxin results in a characteristic cessation of feeding, followed by general paralysis or lack of movement, and death occurs within a few hours or days [11].

The present study was intended to determine how infection with *P. hermaphrodita* (Schneider) changes the response of the snail *H. aspersa* when exposed to two toxicants: Cadmium, and Bt toxin expressed by transgenic maize MON810. We conducted two types of experiments. In the first, we exposed the snails to cadmium (via food and soil) and to nematodes simultaneously. In the second, we exposed the snails to Bt maize material and to nematodes at the same time.

MATERIALS AND METHODS

Animals and soil

Juvenile snails (age, one month; wt, ~1 g) were obtained from a stock culture kept in the Department of Environmental Biology, University of Franche-Comte, Besançon, France. This is the stage recommended by the International Standardization Organization [12] for standard ecotoxicological tests on snails, because at this stage, their growth is the fastest.

The soil was from Foulum, Denmark, and was a loamy sand soil (28% coarse sand, 34% fine sand, 23.2% silt, 8.3% clay) containing 6.4% organic matter and pH 5.7. After the water-holding capacity (WHC) of the soil was measured, the soil moisture was maintained at 60% WHC. At the beginning of the experiments, the soil was mixed with distilled water or given a solution of cadmium to 50% WHC, and the remaining 10% of the 60% WHC was made up by spiking the soil with solutions containing a given concentration of nematode dauer larvae.

A commercial pack of dauer larvae of *P. hermaphrodita* (Nemaslug) was obtained from Becker Underwood (Littlehampton, UK). To check the number of infective larvae in the product and to prepare solutions for the experimental treatments, a stock solution was prepared in the following manner: The contents of the pack were mixed with 500 ml of tap water in a 1-L, graduated cylinder, then filled with tap water to make up 1 L and aerated vigorously. After the air supply was turned off, 1 ml of the solution was diluted to 100 ml and then mixed by aeration. The number of living infectives (i.e., individual living dauer larvae) was counted in three 0.1-ml subsamples of the diluted stock using a counting chamber. The number of infectives was counted as number of infectives per milliliter (stock solution) = 100·mean count and as number of nematodes per pack (in stock solution) = number of infectives per milliliter·1,000. Thus, the number of infectives in the Nemaslug pack is 10^5-fold the mean number of individuals counted in the chamber.

We verified infection of *H. aspersa* by *P. hermaphrodita* in a preliminary study before the described experiments. We observed that after the death of a snail caused by nematode infection, the entire soft body was eaten, and new generations of nematodes appeared in the shell and its vicinity.

To prepare suspensions of *P. hermaphrodita* for the experimental treatments, stock solution was mixed with tap water to reach concentrations of infectives equal to 0-, 0.25-, 1-, or 4-fold the recommended field dose (300,000 infectives/m^2; dose recommended by Becker Underwood for home gardens and horticulture). The soil placed in experimental boxes was sprinkled with a given solution of nematodes in each separate box. Thus, the snails were exposed to nematodes via soil from the beginning of the experiment.

Influence of P. hermaphrodita *on toxicity of cadmium to snails*

The snails were exposed to nematodes and cadmium simultaneously. Exposure to cadmium was via food and soil at nominal concentrations of 0, 30, 60, 120, or 240 mg/kg in both soil and food. The soil was mixed with a given solution of cadmium or with distilled water to 50% WHC, and the remaining 10% of the total 60% WHC was reached by sprinkling the soil with solutions of the concentration of nematode dauer larvae. Helixal (Philicot SA, Valdahon, France), a product used in commercial snail farming, was the food source. One-hundred grams of Helixal contains 15.7 g of protein, 7.5 g of crude fat, 1.8 g of total cellulose, 31 g of total ash, 10.3 g of calcium, 20,500 IU/kg of vitamin A, 3,900 IU/kg of vitamin D_3, and 39 IU/kg of vitamin E [12].

The amount of solution used for preparation of experimental food was 100 ml per 1 kg dry weight of food. Cadmium chloride salt solutions ($CdCl_2 \cdot 2.5\ H_2O$; GR-pure; Saint Quentin Fallavier, Merck, France) were used to contaminate soil and food. A food blender was used to mix the solutions or distilled water with soil and food.

Each treatment was applied in four replicates of four snails each (80 boxes in total); each group of four snails was kept together in a mouse box with a plastic lid (transparent polystyrene; IFFA-CREDO, BP 0109, 69592 L'Arbresle Cedex, France). A layer of 200 g of wet soil was in the boxes. The snails were fed ad libitum three times a week; the corresponding type of cadmium-spiked or uncontaminated food was offered to the snails in a Petri dish. Feces, uneaten food, and dead snails were removed at each feeding time, and the cages were watered with tap water as needed. The experiment was carried out in an environmentally controlled chamber under a 18:6-h light:dark photoperiod at 20°C and greater than 80% humidity. After four weeks, the snails were weighed and refrigerated for further analyses.

Cadmium concentration analysis

For chemical analyses, only snails that survived the experiment were taken. The soft bodies of all snails from a given replicate were dried at 105°C for 12 h, weighed, and digested in 2 ml of boiling Suprapur nitric acid (65%; Merck, Darmstadt, Germany). After cooling to room temperature, each sample was diluted to 10 ml with deionized water. Samples of food and soil were similarly prepared for analyses. The samples were analyzed for cadmium concentration by graphite-furnace atomic absorption spectrophotometers (Aanalyst 800; PerkinElmer, Boston, MA, USA). When the metal concentrations exceeded the detection range of the furnace method, samples were analyzed by flame atomic absorption spectrophotometers (Aanalyst 800; PerkinElmer). Every run of analysis was accompanied by three blank samples, two samples of standard reference material corresponding to animal tissue (certified cadmium concentration, 0.544 ± 0.017 mg/kg [95% confidence intervals]; bovine liver Reference Material 185R, sample identification 0478; European Commission, Community Bureau of Reference, Gent, Belgium), and two samples of standard reference material corresponding to soil (certified cadmium concentration, 0.35 mg/kg; Chinese Soil 4, Reference Material GBW 07404; Promochem, Wesel, Germany). Recovery values were 79 and 106% for bovine liver and Chinese soil, respectively. The detection limit for cadmium was 0.38 μg/L; it was calculated as three standard deviations for the mean of calibration–blanks measurement.

Influence of P. hermaphrodita *on toxicity of Bt maize to snails*

Snails from each nematode treatment were divided into two groups and fed on maize powder (harvested material [i.e., ensilage], a mix of stems/leaves/cobs sampled in October 2003 and dried at 40°C before crushing). One group was fed on Bt maize (MON810; Monumental variety MEB307) expressing Bt toxin Cry1Ab, and the other group was fed on non-Bt maize (isogenic line of Monumental). The dry maize powder was received from the Department of Crop Physiology and Soil Science Research Center, Danish Institute for Agricultural Sciences, Foulum, Denmark.

Each treatment consisted of five replicates of four snails each (40 boxes in total). Each replicate was maintained as described above for the cadmium toxicity experiment. The snails were fed ad libitum three times a week; maize powder was offered to snails in a Petri dish. Experimental conditions were as described above. After four weeks, the snails were weighed as described, and the experiment was terminated.

Bt toxin concentration analysis

Analysis of Bt toxin concentration was performed with the Quantiplate™ kit for Cry1Ab/Cry1Ac (AP003; Envirologix, Portland, OR, USA). After homogenization with a vortex agitator of 0.5 g of freeze-dried maize powder (stored until analysis at −20°C) in 10 ml of extraction buffer from the kit, incubation for 4 h at room temperature on a rotative agitator, and centrifugation at 4,000 *g* at 4°C for 10 min, the Bt toxin was measured in the supernatant. Detection and quantification limits of the kit were 0.14 μg/kg and 0.25 mg/kg, respectively.

*Toxicity of Bt material to the target species, diamondback moth (*Plutella xylostella*)*

The strength of the Bt toxin in the materials used was checked with larvicidal activity tests performed on larvae of *P. xylostella*, a species known to be very sensitive to Bt toxin [13]. The eggs used to establish the culture of *P. xylostella* were obtained from the Institut National de la Recherche Agronomique, Equipe Entomologie et Lutte biologique (Antibes, France). Freshly hatched larvae were fed cabbage leaves. After emerging from pupae, adults were put in a mouse box with a sugar solution as a source of food and water, along with a piece of cabbage leaf on which to lay eggs. Last-stage larvae hatched from the eggs were used to check Bt toxin activity. Larvae were exposed to either untreated cabbage leaves or cabbage leaves coated with a solution containing the Bt or non-Bt maize formulation used in the present study at a concentration of 0.1 g of dry material per 1 ml of distilled water. Three milliliters of the solution were spread on 5 g of cabbage leaves and then offered to 10 last-stage moth larvae. Mortality was checked after 24 h. Each treatment was replicated three times.

Table 1. Cadmium concentrations (mg/kg dry wt) in food and soil in experiments on the influence of *Phasmarhabditis hermaphrodita* infection on the toxicity of cadmium to the snail *Helix aspersa*

Nominal concentration	Soil			Food		
	n	Mean	SE[a]	*n*	Mean	SE
0	3	0.292	0.0333	3	0.615	0.0783
30	2	33.58	2.685	3	19.18	0.074
60	3	49.05	5.623	3	42.88	7.368
120	3	119.7	7.12	3	100.2	16.10
240	3	203.6	5.66	3	250.0	44.06

[a] SE = standard error.

Calculations and statistical analysis

For statistical analysis of the growth rate, the growth coefficient (*GC*) was calculated for each replication (average for all snails in the replication) according to the following equation:

$$GC = \frac{(M_t - M_0)}{M_0} \cdot 100$$

where M_t is the mass (g) of the snail at time t and M_0 is the mass (g) of the snails at time zero [12].

All the data obtained were checked for normality and homogeneity of variance. For the experiment with transgenic maize, growth coefficients were compared by two-way analysis of variance with nematode and maize treatment as factors.

In the statistical analyses, we used nominal instead of measured cadmium concentrations. A linear relationship was observed between the nominal and the measured cadmium concentrations (Table 1) in both soil and food ($r^2 = 0.979$ and 0.841, respectively; $p < 0.0001$ for both).

For the growth rate data from the cadmium experiment, we performed nonlinear-regression analyses using the following equation:

$$y = GC_0 \cdot e^{(Cd \cdot a)}$$

where GC_0 is the intercept, *Cd* is the nominal cadmium concentration, *a* is the slope, and *e* is the base of the natural logarithm. The statistical significance of the differences was estimated on the basis of asymptotic confidence intervals [14].

The influence of nematode treatment on the uptake of cadmium in snails was analyzed as follows: Data were first checked for normality, homogeneity of variance, and linearity

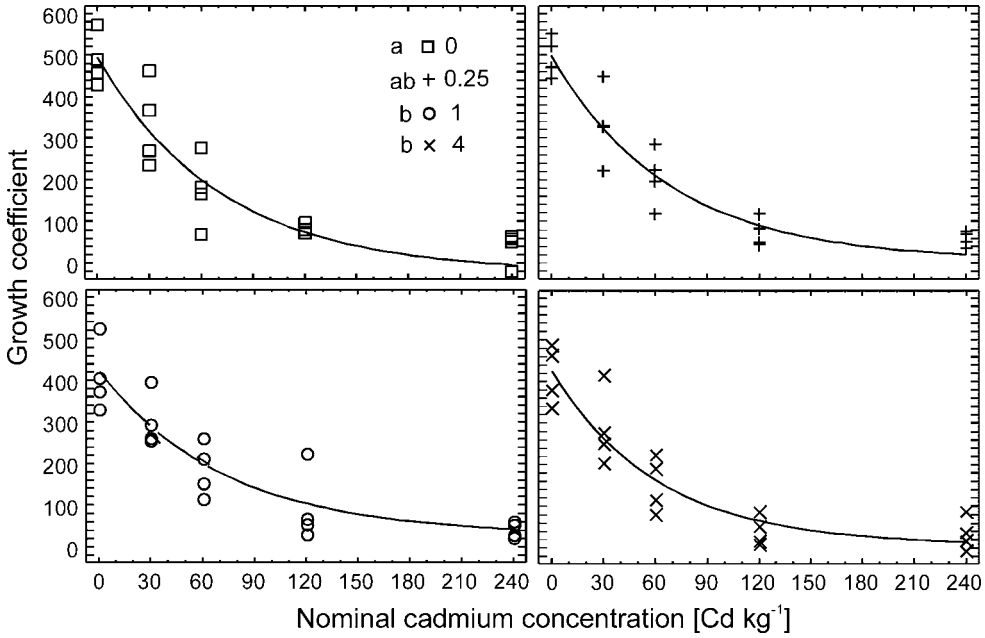

Fig. 1. Influence of nematode infection on the effect of cadmium on growth in the snail *Helix aspersa*. Values with different letters differ significantly at $p < 0.05$. Symbols denote nematode doses; key shows amount of dose as a multiple of the recommended field dose.

of the relationship between the cadmium concentration in snails and the nominal cadmium concentration. Linear-regression lines were then compared using dummy variables (Statgraphics Package; StatPoint, Herndon, VA, USA). The procedure was performed in two steps. First, the significance of the overall differences between intercepts and slopes across all treatments was tested. After significant differences were detected, pairwise comparisons were performed to find differences between treatments.

For both experiments (with maize and cadmium), the recorded time to death was analyzed and compared among treatments with survival analysis. Comparison of survival curves were conducted with logrank and Wilcoxon tests. For the cadmium experiment, the proportion of dead snails in each treatment also was analyzed. Because we expected a linear relationship between the proportion of dead snails and the cadmium concentration, we intended to use regression analysis at the first instance and then to compare regression lines with nematode dose as a factor. However, because clear linear relationships were observed in only a few cases, the results on survival were finally analyzed using the two-way analysis of variance with nematode cadmium and concentration dose as factors. If significant differences were detected, means were separated by the Tukey honestly significant differences (HSD) test. To obtain normal distribution, the data were arcsinus(square root) transformed [14].

Outcomes of bioassays with *P. xylostella* were analyzed using one-way analysis of variance with maize treatment as the factor. To obtain normal distribution, the data were arcsinus(square root) transformed [14].

RESULTS

Influence of P. hermaphrodita *on toxicity of cadmium to snails*

For the effects of both factors on growth in the snail *H. aspersa*, comparison of the asymptotic confidence intervals revealed statistically significant differences between intercepts ($p < 0.05$). The two highest nematode doses differed significantly from the control treatment ($p < 0.05$) (Fig. 1).

In the case of cadmium concentrations in snails, comparison of the regression lines revealed statistically significant differences between intercepts ($p > 0.05$) (Fig. 2). The slopes did not differ, so analyses assuming equal slopes were performed (Statgraphics Package). Statistical analysis showed differences only between the highest nematode dose and the remaining treatments ($p < 0.05$) (Fig. 2).

Comparison of time to death and survival curves did not reveal any influence of nematodes or cadmium on snail survival, but the effects of both factors on the proportion of dead animals were statistically significant, with nematode treatment at $p = 0.0004$ (Fig. 3 and Table 2) and cadmium treatment at $p = 0.00001$ (Fig. 3 and Table 2). The interaction between factors ($p = 0.002$) (Fig. 3 and Table 2) was most evident for the treatment with 120 mg/kg of cadmium; only the nematode-infected snails died of metal toxicity (Fig. 3 and Table 2).

Influence of P. hermaphrodita *on toxicity of Bt maize to snails*

The content of Bt-toxin Cry1Ab was determined to be 16.82 ± 0.8 mg/kg (mean ± SD) in the Bt (MEB307) maize powder. Survival of the diamondback moth on Bt plant material differed significantly from its survival in the control and non-Bt treatments ($p < 0.05$). Average mortality of larvae on the Bt and non-Bt plant material just after 24-h exposure was 40 and 7%, respectively, confirming the larvicidal activity of Bt maize.

The effects of both factors on growth in the snail *H. aspersa* were statistically significant, with nematode treatment significant at $p = 0.006$ (Fig. 4) and maize treatment significant at $p = 0.0091$ (Fig. 4). No interaction was found between treatments ($p = 0.4$). Following exposure to both Bt and non-Bt maize, the growth coefficient of the snails decreased with increasing numbers of nematode larvae (Fig. 4).

No difference was detected between control treatments, in-

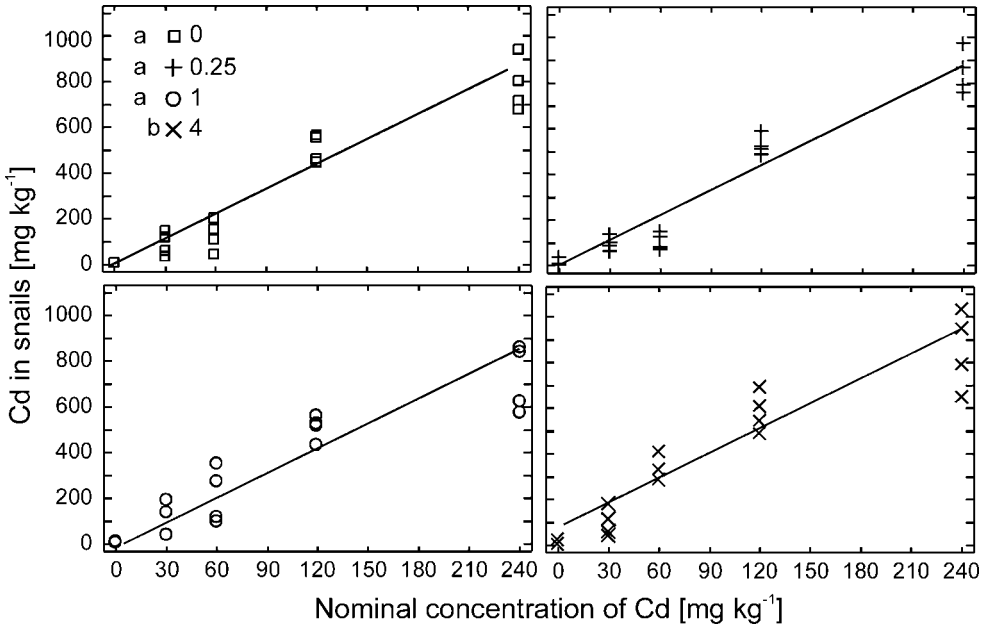

Fig. 2. Influence of nematode infection on cadmium uptake by the snail *Helix aspersa*. Values with different letters differ significantly at $p < 0.05$. Symbols denote nematode doses; key shows amount of dose as a multiple of the recommended field dose.

dicating that Bt maize was not harmful to the snails in the absence of nematodes. No significant difference was found in growth rates between control (i.e., no nematodes) snails fed Bt or non-Bt maize infected up to the field dose. Growth of snails infected with fourfold the field dose and fed non-Bt maize also was not significantly different from that of the controls. However, the growth rate of snails infected with fourfold the field dose and fed Bt maize was significantly lower than that of the control snails (Tukey HSD, $p > 0.05$) (Fig. 4). Growth rates of snails fed Bt maize and infected with 0.25-, 1-, and 4-fold the field dose did not differ from each other (Tukey HSD, $p > 0.05$) (Fig. 4).

The infected snails fed Bt maize had a slightly lower growth rate than animals not exposed to Bt toxin, especially when infected with the highest nematode dose (Fig. 4), but the interaction between treatments was not significant ($p = 0.4$) (Fig. 4). It also is worth mentioning that snails fed Helixal grew faster than the snails fed maize—even those snails fed conventional maize (Figs. 1 and 4).

Comparison of time to death and survival curves did not reveal any influence of nematodes or Bt maize material on snail survival. None of the snails died in that experiment.

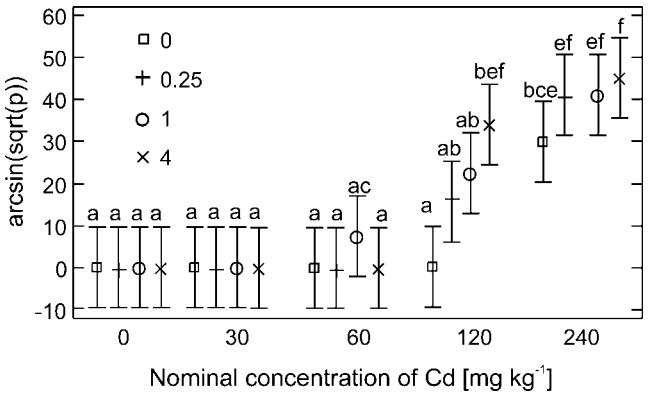

Fig. 3. Influence of nematode infection and exposure to cadmium on the proportion of dead snails (p). Mean values and 95% Tukey honestly significant difference (HSD) intervals are shown. Values with different letters differ significantly at $p < 0.05$ (Tukey HSD test). Symbols denote nematode doses; key shows amount of dose as a multiple of the recommended field dose. Note that the y axis is arcsinus(square root)-transformed to arcsin(sqrt(proportion of dead snail)).

DISCUSSION

The present study has shown that infection with parasitic nematodes can exacerbate the harmful effects of other stressors on *H. aspersa*. The most notable results were obtained from the cadmium experiment: Infected snails were more sensitive to cadmium exposure, and they also accumulated the highest

Table 2. Proportion of dead snails in experiments on the influence of *Phasmarhabditis hermaphrodita* infection on the toxicity of cadmium to the snail *Helix aspersa*[a]

Nematode dose[b]	Cadmium concentration (mg/kg)				
	0	30	60	120	240
0	0	0	0	0	0.25
0	0	0	0	0	0.25
0	0	0	0	0	0.25
0	0	0	0	0	0.25
0.25	0	0	0	0	0.25
0.25	0	0	0	0	0.50
0.25	0	0	0	0.25	0.50
0.25	0	0	0	0.25	0.50
1	0	0	0	0.25	0.50
1	0	0	0	0.25	0.25
1	0	0	0	0	0.25
1	0	0	0.25	0.25	0.75
4	0	0	0	0.25	0.50
4	0	0	0	0.25	0.50
4	0	0	0	0.25	0.50
4	0	0	0	0.50	0.50

[a] Data for each replicate are given.
[b] The nematode dose is given as the fold value relative to the recommended field dose of a commercial nematode application for agricultural use.

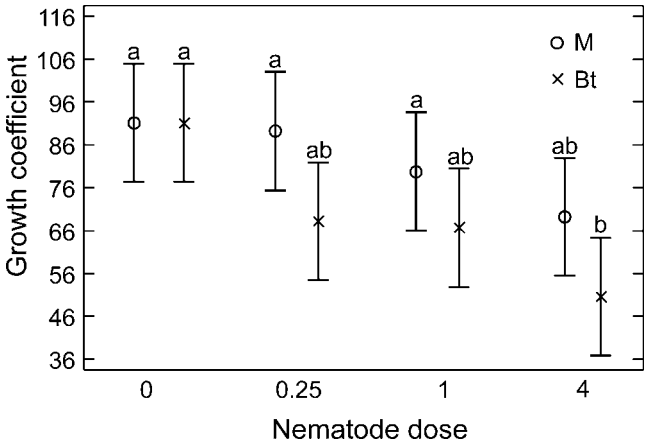

Fig. 4. Influence of infection with nematode larvae on the effect of Bt (expressing *Bacillus thuringiensis* toxin) maize on growth in the snail *Helix aspersa*. Mean values and 95% Tukey honestly significant difference (HSD) intervals are shown. Values with different lowercase letters differ significantly at $p < 0.05$ (Tukey HSD test). Key shows amount of dose as a multiple of the recommended field dose. Bt = Bt maize; M = non-Bt maize.

amount of cadmium. In contrast to our results, Sures et al. [15] found that rats infected with the cestode *Hymenolepis diminuta* had a lower concentration of lead compared with that of uninfected individuals, probably because the nematodes accumulated a high level of lead (i.e., they contained several-fold the level of this metal found in their hosts). We did not analyze cadmium concentrations in *P. hermaphrodita*, so we cannot correlate the metal concentrations in the parasites and their hosts. One possible explanation of our results is that the severely infected snails were less able to eliminate cadmium. On the other hand, mollusks infected by nematodes exhibit reduced feeding [8], and this should result in lower levels of accumulation of cadmium. More research will be required to clarify these results.

In the case of maize treatments, the Bt toxin was only toxic to snails when the snails also were compromised by nematode infection at the highest dose. Koppenhöfer and Kaya [16] obtained similar results in a study with two species of grubs, *Cyclocephala hirta* and *Cyclocephala pasadenae*. Those authors showed that simultaneous exposure to combinations of a commercial formulation of *B. thuringiensis* (Javelin) and entomopathogenic nematodes (*Heterorhabditis bacteriophora* or *Steinernema glaseri*) had additive effects on the morality of the grubs. In another study regarding the influence of Bt toxin on *H. bacteriophora* and *S. glaseri*, Kaya et al. [17] found that both nematode species could survive well in *B. thuringiensis* formulations.

We found the clearest influence of *P. hermaphrodita* infection on either Bt or cadmium toxicity to *H. aspersa* when snails were treated with nematode doses exceeding the dose recommended for use against slugs. This can be explained by documented resistance of *H. aspersa* to *P. hermaphrodita* [9]. A study by Charwat et al. [18], however, showed helicid snails to be very sensitive to infection with this species of nematode, and in the present study, we exposed juvenile snails with a mass of no greater than 1 g, as recommended by Wilson and Gaugler (http://www.nysaes.cornell.edu/ent/biocontrol/). Furthermore, a study by Jaworska and Tomasik [19] demonstrated that some metal ions (including cadmium ions) were toxic to the nematodes *H. bacteriophora* and *Steinernema carpocapsae* and, thus, lessened the infectivity of nematodes in the wax moth caterpillar *Galleria mellonella*. Research concerning metal toxicity to *P. hermaphrodita* is lacking; in our experiment with cadmium, the infectivity of nematodes seems to be enhanced by exposure to this metal. In any case, the response of *H. aspersa* to *P. hermaphrodita* needs more detailed study, especially because little scientific data are available regarding the infection of snails with *P. hermaphrodita* and concerning the influence of infection with this parasitic nematode on the susceptibility of snails to other stressors.

Acknowledgement—The authors are grateful to Ryszard Laskowski and to three anonymous reviewers for their valuable comments. Michael Jacobs helped to edit the text. The study was partly supported by the European Union (EU) project QLRT-2001-01666, Soil Ecological and Economic Evaluation of Genetically Modified Crops (ECOGEN), and partly by the EU-integrated project NoMiracle (Novel Methods for Integrated Risk Assessment of Cumulative Stressors in Europe) contract 003956 under the EU-theme ''Global Change and Ecosystems'' topic ''Development of risk assessment methodologies,'' coordinated by Hans Løkke at the National Environmental Research Insitute, DK-8600 Silkeborg, Denmark.

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