

Predator–Prey Relationships in a Two-Species Toxicity Test System

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In a two-species toxicity test system survival and reproduction of both the predator *Hypoaspis aculeifer* (Gamasida) and the prey *Folsomia fimetaria* (Collembola) were studied after 21 days of residual exposure to a soil contamination of the insecticide dimethoate. Additional experiments were run to analyze which species–species and compound–species relationships determine the outcome of this two-species experiment. Number of adult *F. fimetaria* were reduced by both predation and dimethoate exposure, whereas mites preyed less efficiently on adults than on juveniles. At 0.357 mg dimethoate/kg soil, numbers of juvenile *F. fimetaria* were mainly reduced by predation on adults and juveniles. At 0.7 mg/kg, an additional dimethoate effect was found, which was attributed to an effect on the reproduction of *F. fimetaria*, mainly due to lethality of adults. It was reasoned that lethal effects on juvenile springtails are less important. Adult *H. aculeifer* was not affected by dimethoate exposure, whereas numbers of juvenile *H. aculeifer* demonstrated a decline only at the highest concentration of 0.7 mg/kg. It is hypothesized that this latter effect is possibly due to food depletion caused by a decreased availability of prey, rather than to the lethal effects of dimethoate on juvenile mites. Such a secondary effect of a pesticide application could not have been derived from a single-species toxicity experiment and demonstrates the additional value of a two-species toxicity test system. © 1997 Academic Press

INTRODUCTION

In recent years, many single-species test systems have been developed for soil toxicity testing of potential hazardous chemicals. One of the major drawbacks of these systems is that they do not account for species–species interactions as found in nature. These interactions may have a large influence on the response of an ecosystem to toxicant exposure, which was not to be expected based on the outcome of a single-species laboratory toxicity test. For example, resurgence of English grain aphids (*Sitibion avenae* F.) was found by Lowe and Benevicius (1981) in cereal fields after pesticide application, due to suppression and slow recovery of natural enemies predated on the pest population. Edwards and Thompson (1973) reported simi-

lar increases in numbers of springtails due to a decrease in numbers of predacious mites after pesticide application.

In the present study, survival and reproduction of both predators and prey were investigated after 21 days of residual exposure to soil contamination. For this purpose, a two-species toxicity test system was developed which consists of a 21-day reproduction and survival experiment in soil with a predator (mite) and prey (Collembola) species. *Hypoaspis aculeifer* Canestrini (Gamasida: Laelapidae) and *Folsomia fimetaria* L. (Collembola: Isotomidae) were used as predator and prey species, respectively. Dimethoate was used as test chemical.

The aim of this paper is to determine the effect of a pesticide on the predator–prey relationship. This was done on the basis of a model which is illustrated in Fig. 1. All entities which are present in the test system are presented in separate boxes. Some of the processes going on in the toxicity test were analyzed individually in four separate experiments, in either the absence or the presence of the pesticide. The results of all five experiments were used to determine which steering processes in the predator–prey relationship were mainly affected by dimethoate.

MATERIAL AND METHODS

Test Animals

Both *H. aculeifer* and *F. fimetaria* specimens were obtained from synchronized cultures, which were fed *Folsomia candida* Willem (Collembola: Isotomidae) and baker's yeast, respectively. Both cultures were bred on a gypsum–charcoal substrate (Paris plaster) at 20°C. Synchronism was obtained by changing the substrate every 3 days for stimulation of oviposition. Ages of test animals are given in the description of each experiment.

Test Soil

The soil used in the experiments was defaunated sandy loam. The soil composition is given in Table 1. Defaunation was obtained by alternately drying and freezing of the soil (Krogh, 1995a). At preparation, the dried test soil was moistened and inoculated with soil extract (0.154 ml/g) (Krogh, 1995a). Mixing of dry soil and moisture was done with a spatula in a beaker, whereafter the test soil was incubated overnight in the hood. On day 0, the evaporated amount of

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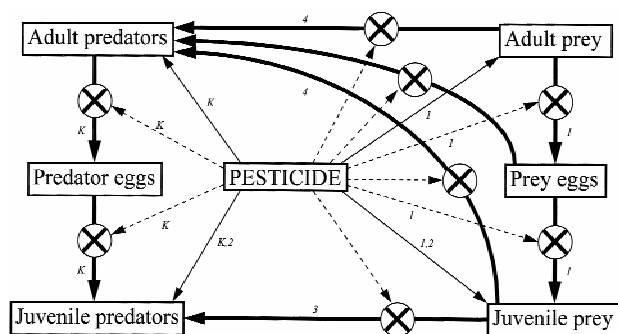


FIG. 1. Entities and their mutual relationships which are present in the two-species toxicity test system after 21 days. The bold arrows indicate the expected flow of matter between the entities (i.e., predation, egg production, and egg hatching). The valves in these arrows indicate that the flows are characterized by a certain rate. The narrow arrows indicate the direct (solid arrow) and indirect (dashed arrow) effect of the pesticide. Characters refer to the separate experiments described in the present paper (experiments 1–4) or by Krogh (1995a) (K) which studied the specific interactions or effects. The entire system was studied in experiment 5.

water was compensated for with demineralized water and the soil was transferred to the microcosms.

Test Chemical

Dimethoate (*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithionate), an organophosphate inhibitor of cholinesterase, was used as the test chemical. It is a broad-spectrum insecticide with pronounced effects on the arthropod fauna at the recommended dose level (Frampton, 1988; Powell *et al.*, 1985; Vickerman and Sunderland, 1977). Dimethoate (Chemnova Argo A/S, 400 g/l EC) was dissolved in the soil inoculate and was added to the soil in an exponential range of concentrations, which is given in the description for each experiment.

Microcosms and Extraction

“In soil” experiments were performed in a cylindrical plexiglass microcosm (inner diameter, 6 cm; height, 5.5 cm). The bottom of the microcosm consisted of a net (mesh width, 1 mm). Evaporation of soil moisture was prevented by a plastic lid on the bottom and the top of the microcosms. After an experiment, surviving animals and juveniles produced were extracted from the microcosms by high-gradient extraction (MacFadyen type). Extraction lasted 48 hr and started at 25°C. Temperature increments were 5°C/12 hr. Extracted animals were collected in a plastic container with a Paris plaster substrate at 2°C. After extraction, the containers were frozen at –20°C and the extracted animals were counted later. Average extraction efficiency for (juvenile) *F. fimetaria* was checked to be 94.0 (SEM = 2.04; $n = 4$) of 100 specimens added to the soil initially.

Experiments

Experiments 1, 2, and 5 were performed in soil in a microcosm, and experiments 3 and 4 were performed on a Paris plaster substrate in a plastic container (diameter, 7 cm; height,

4 cm). All experiments were carried out in a climate room at 20°C.

1. Single species test on *F. fimetaria* adults. The goal of experiment 1 was to determine the effect of residual pesticide exposure in soil on reproduction and survival of adult prey. A single species test with predator adults has been described by Krogh (1995a).

Soil (26 g dry wt) was prepared on day –1. Treatments consisted of a control and of five concentrations ranging from 0.18 to 0.70 mg a.i./kg dry soil. Each treatment was carried out fourfold. On day 0, 25 adult *F. fimetaria* (16–19 days old) were added to the soil. On days 0 and 14, baker’s yeast (15 mg) was added as a food source for the Collembola. On day 21 surviving adults were extracted from the soil, together with their reproductive output.

2. Single species test on *F. fimetaria* and *H. aculeifer* juveniles in fresh or aged soil contamination. The goal of experiment 2 was to determine the survival of juveniles in either a fresh or an aged soil contamination. Earlier experiments found that *F. fimetaria* and *H. aculeifer* start hatching 10 and 7 days (respectively) after the start of a single-species experiment. Therefore, differences between age of contamination (fresh-aged) were chosen to be 10 and 7 days. Together with a test period of 11 and 14 days, this is comparable to the 21-day single-species reproduction test.

On day 0, 25 juvenile *F. fimetaria* (0–3 days old) were placed in soil (26 g dry wt) prepared on either day –1 (fresh) or on day –11 (aged). Treatments consisted of a control and of five concentrations, ranging from 0.18 to 0.70 and from 0.36 to 1.4 mg a.i./kg dry soil for the fresh and the aged soil contamination, respectively. Each treatment was carried out fourfold. Baker’s yeast (15 mg) was added as a food source for the Collembola on day 0. On day 11, surviving juvenile *F. fimetaria* were extracted from the soil.

The experiment was repeated with 10 juvenile (0–3 days old) *H. aculeifer*. On day 0, they were placed in soil prepared on either day –1 (fresh) or day –8 (old). On days 0 and 7, a surplus of juvenile *F. Candida* was added as a food source for the mites. On day 14 surviving juvenile *H. aculeifer* were extracted from the soil.

3. Consumption study. The goal of experiment 3 was to determine the maximum and the actual predation rate.

The first part of the experiment was carried out on a Paris

TABLE 1
Soil Composition of the Sandy Loam Soil Used in This Study

Organic fraction (%)		Mineral fraction (%)			
Total organic carbon	Humus (organic matter)	Clay (<2 μm)	Silt (2–20 μm)	Sand (20–200 μm)	Course sand (0.2–2 mm)
1.6	2.7	10.6	14.5	45.8	26.4

plaster substrate. On day 0, a surplus of 200 juvenile *F. fimetaria* was fed to 10 juvenile *H. aculeifer*. On days 1–3, predation of the Collembola was counted under anesthetization by CO₂. To start each new day of testing with 200 juvenile prey, the numbers of Collembola consumed were replenished daily for each container. On day 4 only predation was scored and the experiment was terminated. The age of the Collembola added daily to the containers was 0–3 days. The age of the mites was 0–3 days at the start of the experiment.

The second part of the experiment was carried out in a microcosm in soil (26 g dry wt) inoculated with 4 ml inoculate. Two hundred juvenile *F. fimetaria* and 10 juvenile *H. aculeifer* were added to each microcosm. After 24 hr the test was terminated and microcosms were extracted. This test was carried out fourfold and was repeated for 3 days. To determine the rate of predation the 48 hr of extraction, a control group of four microcosms was prepared and immediately set to extraction.

4. Catchability study. The goal of experiment 4 was to determine whether the predators indicate a preference for either adult or juvenile prey.

Adult and juvenile *F. fimetaria* were fed in seven different ratios (0:100, 20:80, 40:60, 50:50, 60:40, 80:20, and 100:0) to adult *H. aculeifer* on a Paris plaster substrate. On day 0, 100 *F. fimetaria* individuals (adult or juvenile), 10 adult female, and 5 adult male *H. aculeifer* individuals were added to each container. On days 1 and 2, consumption of the Collembola was counted under anesthetization with CO₂. Numbers of Collembola consumed were replenished daily to ensure that each test day was started with the same ratio of adult vs juvenile prey. On day 3 only predation was counted and the experiment was terminated. The experiment was carried out in duplicate. The ages of the juvenile and the adult Collembola added daily to the containers were 0–3 and 16–21 days, respectively. The age of the mites was 27–30 days at the start of the experiment.

The same experimental design was used in a test with 400 prey individuals. Three ratios of adult vs juvenile Collembola were tested, i.e., 100:300, 200:200, and 300:100. The ages of the juvenile and adult Collembola added daily to the containers were 0–5 and 15–20 days, respectively. The age of the mites was 16–19 days on day 0.

5. Two-species toxicity experiment. The goal of experiment 5 was to determine the overall effect of dimethoate on the predator–prey relationship. The results of the four previous experiments were used to interpret the outcome of this overall experiment.

On day 0, 100 adult *F. fimetaria* and 10 adult female and 5 adult male *H. aculeifer* were added to each microcosm, containing 52 g (dry wt) of moistened and inoculated soil. All animals were 16–19 days old at the start of the experiment. On days 0 and 14, 15 mg of baker's yeast was added as a food source for the Collembola. On day 21, surviving adults (Collembola and mites) together with their reproductive output were extracted from the soil.

Two different versions of this experiment were carried out:

(A) The “interrupted experiment” consisted of a control and two concentrations, i.e., 0.357 and 0.70 mg a.i./kg dry soil. Each treatment was carried out 12-fold and extraction of four replicates took place not only on day 21, but also on days 9 and 14 in order to provide insight in the processes in the microcosms during the 21-day test period.

(B) The “10 replicates experiment” consisted of a control and two concentrations, i.e., 0.357 and 0.70 mg a.i./kg dry soil. Extraction took place on day 21 and, for reasons of reproducibility, each treatment was carried out 10-fold.

Data Analysis

LC₅₀'s and EC₅₀'s were calculated by nonlinear regression analysis based on two models (Model I and II) described by Lacey and Mallett (1991). In each case the best fitting model (ANOVA) was adopted. NOECs and concentrations causing responses which differ significantly from the control ($P = 0.05$) were calculated from square root transformed response data by means of the program TOXSTAT (Gulley *et al.*, 1988): Williams' test was executed for normally distributed (χ^2 test) results, demonstrating homogeneity of variance (Hartley's test). NOECs of results failing one or both tests were determined using the nonparametric Kruskal–Wallis test. Differences in the rate of predation between experimental days were tested by Tukey's test ($P = 0.05$). Preference for predation on adults or juveniles was tested by the log likelihood ratio test (G -test; $P = 0.005$). Linear and nonlinear regressions were performed using the PROC REG and PROC NLIN procedures of the SAS statistical package (SAS Institute Inc., 1988).

RESULTS

Single-Species Test on *F. fimetaria*

Average production of *F. fimetaria* was significantly lower (William's test) at the two highest dimethoate concentrations tested (Fig. 2). Average survival of adult *F. fimetaria* indicated a decline with increasing concentrations (Figure 3), but no significant differences from the control were found (Kruskal–Wallis). Table 2 presents EC₅₀ values and NOECs for reproduction and survival.

In the control microcosms, mean reproduction was 205.5 (SEM = 93.0; $n = 4$) and mean survival was 15.25 (SEM = 4; $n = 4$) of the 25 adults initially added to each test container.

Single-Species Test on *H. aculeifer* and *F. fimetaria* Juveniles in Fresh or Aged Soil Contamination

In both aged and fresh soil contaminations, juvenile *F. fimetaria* appeared to be much more sensitive to dimethoate than juvenile *H. aculeifer*. Within the range of concentrations tested, dimethoate did not affect survival of juvenile *H. aculeifer*, neither in the aged nor in the fresh soil contamination. Survival of juvenile *F. fimetaria* was severely affected at the highest concentrations tested. The fresh soil contamination appeared to be more toxic than the aged soil contamination (Fig.

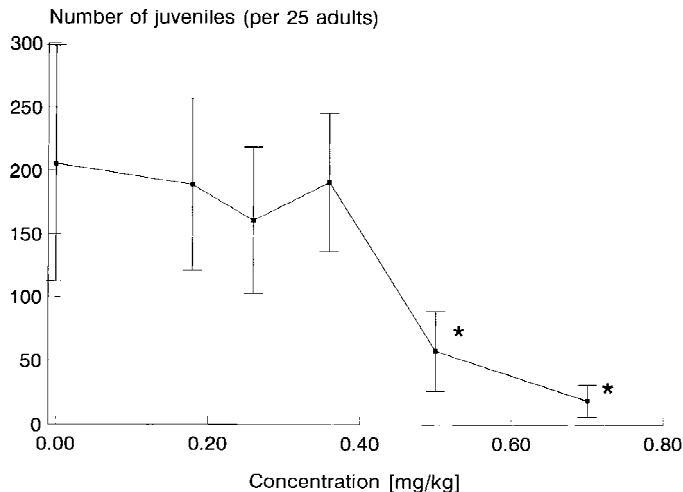


FIG. 2. Mean reproduction of *F. fimetaria* (number per 25 adults) after 21 days of residual exposure to a soil contamination of dimethoate (experiment 1). Significant differences from the control (William's test; $P = 0.05$) are indicated with an asterisk.

4 and 5), resulting in lower LC_{50} and NOEC values (Table 2). Based on the difference between both LC_{50} 's and assuming an exponential decay of dimethoate in soil, a biologically determined half-life time of $t_{1/2} = 12.5$ days was calculated from this experiment.

In the control microcosms, mean survival in the freshly prepared and in the aged soils was 23.3 (SEM = 1.03; $n = 4$) and 16.7 (SEM = 4.37; $n = 4$), respectively, of the 25 specimens initially added to each test container.

Consumption Study

Predation on Paris plaster did not differ significantly between the test days (Tukey's test). Therefore, the duplicate observations of the 3 test days were pooled and considered as six replicates. By means of a linear regression on the cumulative consumption (Fig. 6), an average daily consumption of 8.0 juvenile prey per juvenile predator was calculated.

In soil, an average consumption of 68.0 was found in juvenile prey (SEM = 3.98; $n = 12$) per 10 juvenile predators after a test period of 24 hr and an extraction period of 48 hr. By means of an ANOVA, this average consumption could not be distinguished from the average consumption of 63.8 (SEM = 9.38; $n = 4$), which was found after only 48 hours of extraction. This relatively high consumption during extraction hampered calculation of an average daily consumption in soil.

Catchability Study

Results of experiment 4 did not differ significantly between the duplicates nor between the different days (Tukey's test). Therefore, all data were pooled per ratio and were considered as six replicates.

In Fig. 7, the relative consumption of adult *F. fimetaria* is presented when 100 or 400 adult and juvenile prey were offered in different ratios to 10 adult female and 5 adult male *H.*

aculeifer. The relative contribution of adult *F. fimetaria* to the diet of the mites appeared to be significantly smaller (G test) than the relative presence of adult *F. fimetaria* in the initial number of Collembola offered to the mites. In other words, juvenile prey were consumed at higher ratios than they were offered to the mites.

Two-Species Toxicity Experiment

Numbers of adult and juvenile *F. fimetaria* and of juvenile *H. aculeifer* in experiment 5A are given in Fig. 8. Numbers of adult mites are not presented, because at all concentrations tested, survival of adult mites was nearly 100%.

Numbers of adult *F. fimetaria* revealed a rapid decline within the first 9 days of the experiment. On days 9, 14, and 21, the survival of adult Collembola decreased with increasing dimethoate concentration (Fig. 8).

At all three concentrations tested, numbers of juvenile *F. fimetaria* were still very low on day 9 (Fig. 8). Control microcosms exhibited a rapid increase between day 9 and 14, whereas the 0.357 mg/kg microcosms demonstrated this increase between days 14 and 21. At the highest concentration of 0.7 mg/kg, number of juvenile Collembola remained very low.

At all three concentrations tested, numbers of juvenile *H. aculeifer* exhibited a regular increase in time (Fig. 8). However, at the highest concentration, a decline was found between Days 14 and 21, resulting in significantly lower numbers on day 21 than in the control (William's test).

Results of the dose-response experiment 5B are presented in Table 3. Both adult and juvenile *F. fimetaria* indicated a significant decrease (Kruskal-Wallis) with increasing concentration, whereas the number of juvenile *H. aculeifer* remained constant. No mortality was found for adult mites.

DISCUSSION

The aim of the separate experiments was to obtain a better understanding of the outcome of a two-species toxicity test

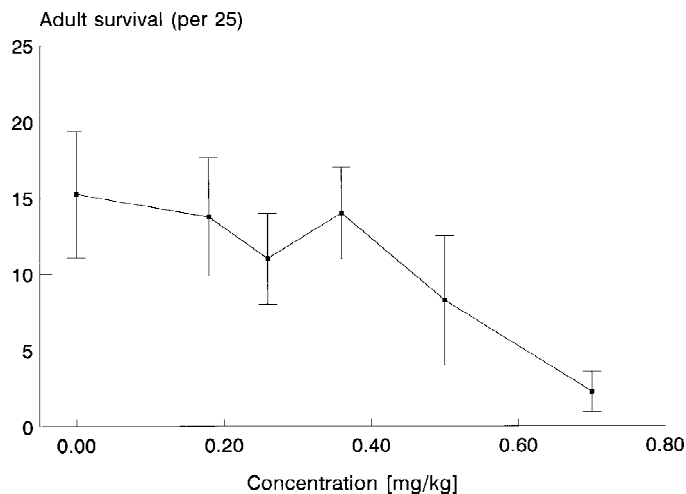


FIG. 3. Mean survival of adult *F. fimetaria* after 21 days of residual exposure to a soil contamination of dimethoate (experiment 1). No significant differences from the control were found (Kruskal-Wallis; $P = 0.05$).

TABLE 2
E(L)C₅₀'s (95% CI) and NOECs (P = 0.05) from Single-Species Toxicity Experiments with Dimethoate and *F. fimetaria*

Experiment	Stage	Exposure time (days)	Day of soil preparation	Effect on	Criterion	Result
1	Adult	21	-1	Reproduction	EC ₅₀	0.48 (0.20-0.75)
1	Adult	21	-1	Reproduction	NOEC	0.36
1	Adult	21	-1	Survival	LC ₅₀	0.54 (0.38-0.70)
1	Adult	21	-1	Survival	NOEC	— ^a
2	Juvenile	11	-1	Survival	LC ₅₀	0.43 (0.38-0.48)
2	Juvenile	11	-1	Survival	NOEC	0.36
2	Juvenile	11	-11	Survival	LC ₅₀	0.75 (0.32-1.18)
2	Juvenile	11	-11	Survival	NOEC	0.71

^a No significant differences from the control were found (Kruskal-Wallis; $P = 0.05$).

system. In this discussion the compound-species and species-species relationships are described separately per test species to explain the outcome of the two-species test system in which both types of relationship take place.

Generally

The biologically determined half life of 12.5 days (experiment 2) is higher than the analytically determined half life of 4.8–9.7 days by Kolbe *et al.* (1991) in three different soil types at 20°C. This difference can be explained by the observation that dimethoate in soil is mainly metabolized into omethoate (Kolbe *et al.*, 1991). Omethoate is known as a broad-spectrum insecticide with pronounced effects on arthropod species (Tomlin, 1994) and contributes to a longer toxic stress for the juvenile springtails in the microcosms.

F. fimetaria Adults

Compound absent, predator absent. In absence of both stress factors, an average number of 15.25 of 25 adult springtails was extracted from the soil after 21 days in experiment 1.

In a similar experiment which lasted 28 days, Krogh (1995b) found an average survival of less than 9 adults out of 25. In both cases, no explanation was found for this relatively low number of adult *F. fimetaria* under control conditions.

Compound present, predator absent. This dose-response curve of adult *F. fimetaria* exposed to dimethoate (Fig. 3) is in good agreement with results from Krogh (1995b) in similar experiments. However, from an experiment carried out in soil it cannot incontrovertibly be concluded that the non-extracted fraction of springtails is actually killed by the dimethoate. As Fábíán and Petersen (1994) described, *F. fimetaria* exhibit uncoordinated motion or even immobilization after residual exposure to dimethoate on Paris plaster. This sublethal effect may also have occurred in the microcosms and consequently may have hampered the Collembola to respond to the heat and moisture gradient in the MacFadyen extractor, so that they were unable to escape from the soil, died by desiccation and were not extracted. Therefore, the LC₅₀ of Table 2 should

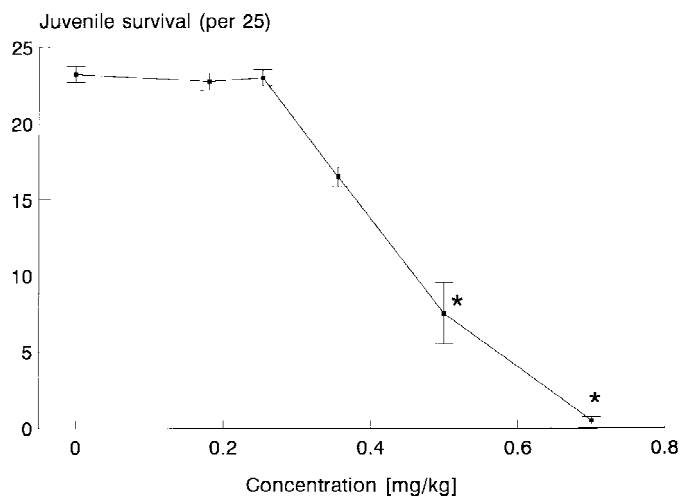


FIG. 4. Mean survival of juvenile *F. fimetaria* after 11 days of residual exposure to a fresh soil contamination of dimethoate (experiment 2). Significant differences from the control (William's test; $P = 0.05$) are indicated with an asterisk.

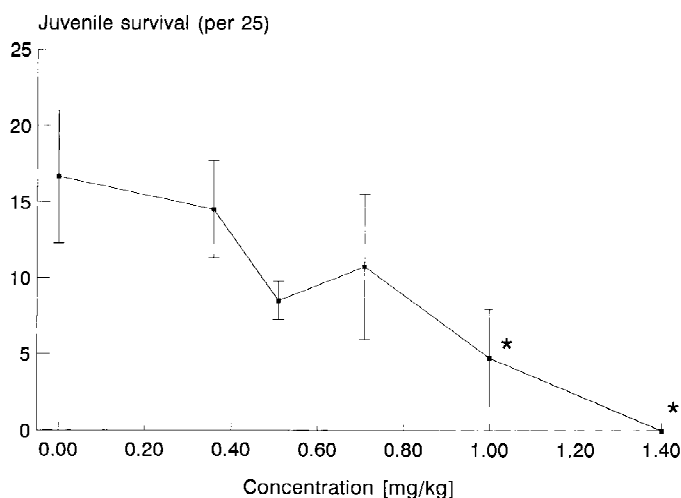


FIG. 5. Mean survival of juvenile *F. fimetaria* after 11 days of residual exposure to a 10-day-old soil contamination of dimethoate (experiment 2). Significant differences from the control (William's test; $P = 0.05$) are indicated with an asterisk.

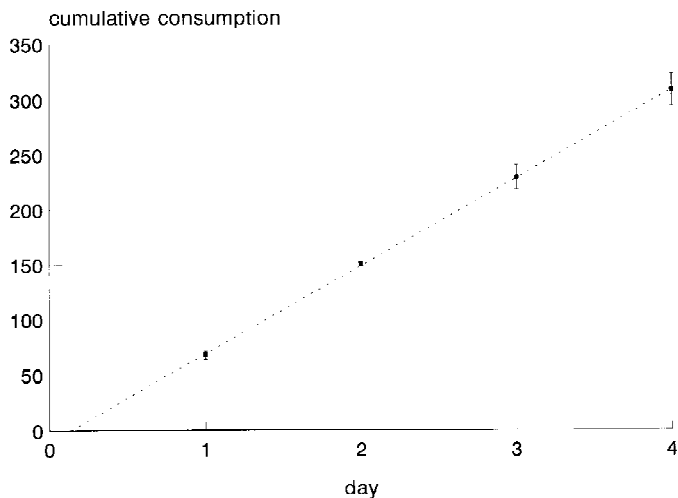


FIG. 6. Cumulative consumption of juvenile *F. fimetaria* by 10 juvenile *H. aculeifer* on Paris plaster substrate. ■, mean ($n = 4$) with SEM; ---, linear regression model.

possibly be interpreted as an immobilization concentration (IC_{50}).

Compound absent, predator present. The fact that adult prey were consumed at lower ratios than they were offered to the mites (Fig. 7; experiment 4) suggests that adult mites prefer juvenile to adult Collembola for prey. In direct observations, the authors noticed that adult springtails were able to escape from the killing grip of an adult mite. Therefore, the "preference" is explained by a higher catchability of juvenile *F. fimetaria* for adult mites.

This result is not directly transferable to the situation in a microcosm. The soil offers many cracks and holes for juvenile prey to escape into, whereas they are too narrow for adult predators to enter. Therefore, it is assumed that this preference

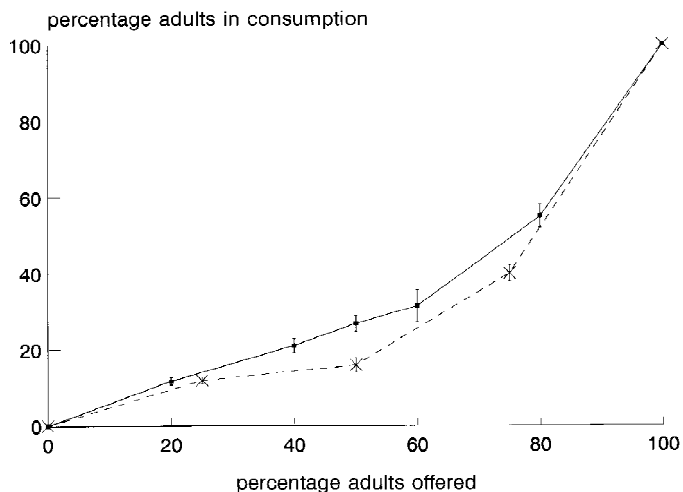
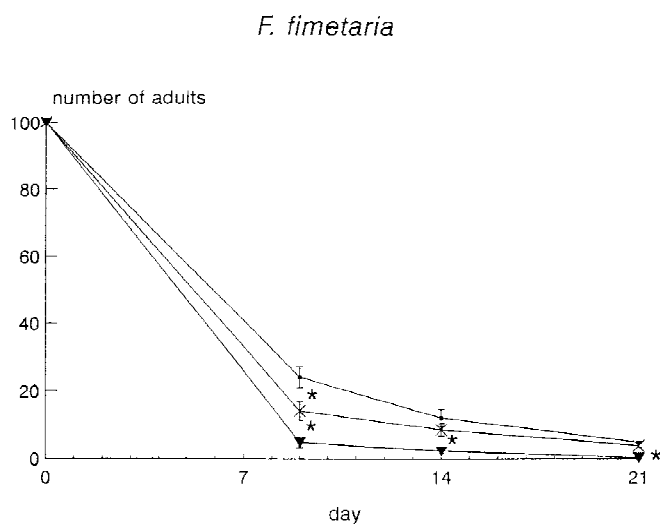
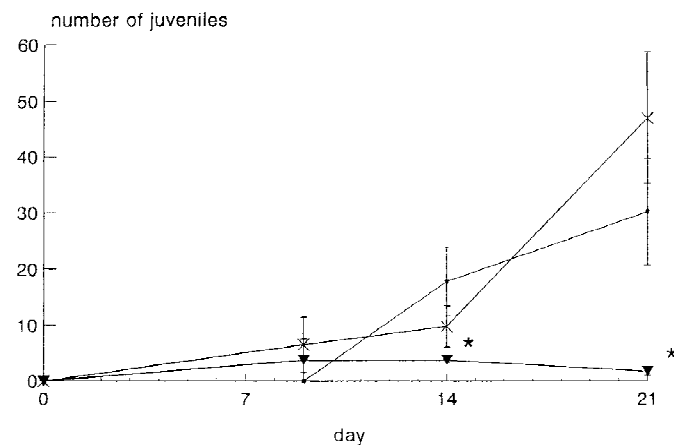


FIG. 7. Composition of the consumption of adult *F. fimetaria* by 10 adult female and 5 adult male *H. aculeifer* on a Paris plaster substrate at different ratios of adult:juvenile prey offered. ■, mean with SEM ($n = 6$; total of prey offered is 100); ×, mean with SEM ($n = 6$; total of prey offered is 400).



F. fimetaria



H. aculeifer

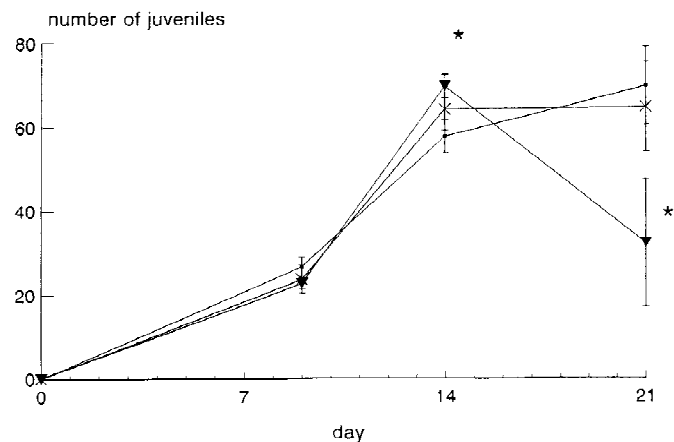


FIG. 8. Average number of adult and juvenile *F. fimetaria* and juvenile *H. aculeifer* (with SEM; $n = 4$) extracted from the microcosms in experiment 5A. Significant differences from the control (William's test; $P = 0.05$) are indicated with an asterisk. ■, control; ×, 0.357 mg/kg; ▼, 0.7 mg/kg.

TABLE 3
Results of the Predator–Prey System after 21 Days
(Experiment 5B)

Species	Number at day 0	Number (SEM) per concentration (mg/kg) at day 21 (n = 10)		
		0	0.357	0.7
Adult <i>F. fimetaria</i>	100	14.1 (4.3)	8.4 (2.4)	0.1* (0.1)
Juvenile <i>F. fimetaria</i>	0	270.5 (71.7)	134 (22.5)	11.5* (3.2)
Juvenile <i>H. aculeifer</i>	0	52.7 (6.3)	69.2 (5.7)	56.5 (5.9)

Note. Significant differences (Kruskal–Wallis; $P = 0.05$) from the control are indicated with an asterisk.

is highly dependent on the density of the soil. The experiment on Paris plaster can then be considered as a “soil-experiment” with maximum soil density. Although no catchability experiment was performed in soil, Krogh (1995a) also found indications of “preference” for juvenile prey in a 21-day microcosm experiment.

The preference described above was based on numbers of prey individuals consumed. Using regressions between dry weight and length of *F. fimetaria* (unpublished results), the relative contribution of both adult and juvenile Collembola to the total biomass consumption was calculated (data not provided). According to these calculations, adult prey contributed more to total biomass consumption than juvenile prey. Furthermore, the food consumption in terms of biomass never reached a maximum limit, not even in the experiment with 400 prey individuals available.

It was concluded that the mites were never satiated and collected as much nutrition as possible, both from juvenile prey which were easier to catch and from adult prey which contributed more to their food demand.

Figure 9 presents the expected 21-day development of the test predator and prey populations in the control microcosms, based on the outcome of similar experiments with *H. aculeifer* and *F. candida* and on observations of the test animals in the cultures. Generally, the trends in the control observations of Figure 8 are similar to the expected population developments of Fig. 9. Closer comparison reveals that the decline of the adult Collembola population by predation took place mainly within the first 9 days (Figure 8) and was not a gradual process as suggested by Fig. 9. Furthermore, final numbers of adult *F. fimetaria* after 21 days were lower than expected based on *F. candida* results. Repetition of the experiment did not result in higher numbers.

Compound present, predator present. In the two-species toxicity experiment (experiment 5), adult *F. fimetaria* declined with increasing dimethoate concentrations (Fig. 8). It was not clear whether this effect should be attributed to directly mortality due to the dimethoate treatment or to immobility and uncoordinated motion which hampers a response to the heat

and moisture gradients in the extractor and/or enhances the catchability of prey organisms. Furthermore, a smaller fraction of the initial number of adult *F. fimetaria* is found after 21 days in the presence of *H. aculeifer* (Fig. 8) than in absence (Fig. 3), at all concentrations tested.

The results confirm that both stress factors (i.e., dimethoate exposure and predation) affected the final number of adult *F. fimetaria* extracted from the two-species toxicity test system.

F. fimetaria Juveniles

Compound absent, predator absent. The low recapture from the control aged soil (experiment 2) was comparable to the recapture of adult springtails (experiment 1), whereas recapture from the fresh soil is higher and meets the expectation. These findings suggest that some parameter changes in the test soils between Days 11 and 21 which hampers survival or extraction of both adult and juvenile *F. fimetaria*. This parameter could not be determined from the experiments.

Compound present, predator absent. The calculated EC_{50} of 0.48 mg/kg of the reproduction experiment (1) is in good agreement with the EC_{50} of 0.3 mg/kg determined Krogh (1995b). The effect on the number of juveniles is assumed to be a combination of the effects on reproduction and on the survival of the juveniles. Almost no reproduction is found in experiment 1 (Fig. 2) at an initial concentration of 0.70 mg/kg (21 days), whereas a 10-day-old soil contamination of this concentration still allows for more than 50% of the juveniles to survive (11 days) (Fig. 5; Table 2). From this result, it is concluded that reproduction is a more sensitive parameter for dimethoate exposure than survival of the juveniles and that an effect of dimethoate on the final numbers of juvenile springtails in the overall microcosm experiment (concentration range,

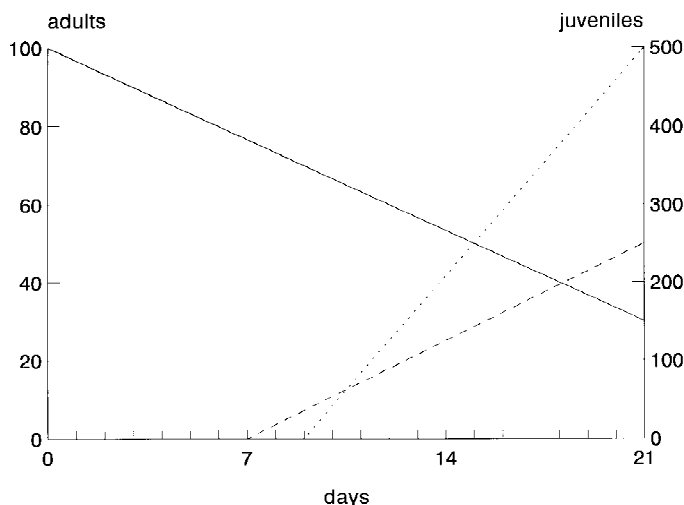


FIG. 9. Expected 21-day development of the test predator and prey populations in control microcosms, based on the outcome of similar experiments with *F. candida* and observations of the test animals in the cultures. —, adult Collembola; ···, juvenile Collembola; ---, juvenile mites.

0–0.70 mg/kg) mainly depends on a reduced reproduction rather than on a reduced survival of juveniles.

Reduced reproduction is mainly attributed to a decline of surviving adults with increasing dimethoate concentrations (cf., Fig. 3 and 2), resulting in a lower number of eggs. Furthermore, dimethoate exposure may affect reproduction by a reduced fecundity and fertility.

Compound absent, predator present. The control observations of Fig. 8 indicate trends similar to those expected for the population developments of Fig. 9. As expected, no juvenile Collembola were found on day 9, and highest hatching rates were found between days 9 and 14. Again, final numbers after 21 days were lower than expected based on *F. candida* results, also after repetition of the experiment.

From experiment 3, an average daily consumption on Paris plaster of 8 juvenile Collembola per juvenile mite was calculated. This consumption rate is in good agreement with results found by Krogh (1995a).

Daily consumption of juveniles in soil is hard to determine from experiment 3, due to the fact that predation during extraction occurred. Predation during extraction may be due to the fact that prey density increases locally at the bottom of the microcosm during extraction, rendering Collembola easier to catch. This undesired process may play an even more important role when dimethoate is present, which may cause immobility and uncoordinated motion of springtails enhancing their catchability. The impact of predation during extraction on the outcome of a 3-week microcosm toxicity experiment needs further investigation.

Though exact determination of the predation rate on juvenile *F. fimetaria* in soil is impossible, some tentative conclusions may be drawn. From Fig. 2 (experiment 1), an average reproduction of 822 (SEM = 372) can be calculated for the 100 adult *F. fimetaria* in the control microcosms of experiment 5. This number is highly overestimated, since no account is taken of predation on the adult springtails and its subsequent effect on their reproduction. Extraction of the control microcosms in experiment 5A on Day 21 yields an average of 69.8 juvenile mites. Assuming that the number of mites linearly increased from 0 on day 7 to 69.8 on day 21 (Fig. 9), an average number of 34.9 juvenile mites is determined to be present during the last 14 days of the experiment. Assuming further that juvenile mites only prey on juvenile prey and with the same predation rate as on Paris plaster (eight prey per mite per day; experiment 3), an average of 14-day predation by the juvenile mites can be calculated of $34.9 \times 8 \times 14 = 3909$ juvenile Collembola. Considering the facts that this number is much higher than the estimated prey reproduction of 822, that juvenile prey are still found in the microcosms after 21 days, and that predation by adult mites (both on adult and juvenile prey) is ignored in the calculation above, it is concluded that the predation rate is much lower in soil than on Paris plaster, probably because of a reduced chance of encounter since prey organisms can hide in

cracks and holes in the soil. This lower predation rate leads to a proportional lower degree of food satiation of *H. aculeifer* in the microcosm experiments.

Compound present, predator present. On days 14 and 21, population densities of juvenile springtails were significantly lower (William's test, $P = 0.05$) at the highest test concentration (0.7 mg/kg) than in the control microcosms (Fig. 8). This finding is comparable to the result at 0.7 mg/kg in the single-species experiment reproduction experiment (Fig. 2). As argued above, this reduction should be attributed mainly to reduced reproduction rather than to juvenile mortality caused by dimethoate. At the intermediate test concentration (0.357 mg/kg), dimethoate treatment did not clearly affect adult survival. However, reproduction per adult at this concentration was much lower in presence of a predator than at the same concentration in absence of a predator (cf. experiments 1 and 5 in Table 4). From these results it is concluded that number of juvenile Collembola was inhibited at 0.357 mg/kg by (enhanced) predation on adults and juveniles or even eggs and at 0.7 mg/kg also by a reduced reproduction due to dimethoate exposure of the adult Collembola and the eggs.

H. aculeifer Adults

Survival of adult mites in the 21-day microcosm experiments was not affected by dimethoate within the concentration range tested (experiment 5). This finding is confirmed by results of Krogh (1995a) who found (significant) mortality only at concentrations ≥ 1.5 mg/kg in comparable experiments.

H. aculeifer Juveniles

As expected, mites hatched earlier than Collembola, with the greatest increase of the juvenile mite population between days 9 and 14 (Fig. 8).

As demonstrated, mites were not satiated in the microcosms, even in the absence of dimethoate. This is confirmed by the low reproductive output of the control microcosms of experiment 5. On day 21 an average of 69.8 juvenile mites were extracted (experiment 5A), which are supposed to have hatched from eggs produced by the 10 adult female mites

TABLE 4
Average Reproduction (21 days) of *F. fimetaria* Juveniles per Adult Initially Present

Concentration dimethoate (mg/kg)	Experiment		
	1	5A	5B
0	8.22	0.30	2.7
0.182	7.55		
0.255	6.42		
0.357	7.63	0.47	1.34
0.50	2.31		
0.70	0.74	0.018	0.12

Note. Experiments were performed in microcosms in the absence (experiment 1) and the presence (experiment 5) of predation.

between days 0 and 14. Thus, an average rate of reproduction of 0.5 juveniles per female per day can be calculated. This is much lower than the average reproduction rate of 2.1 juveniles per female per day which can be determined for satiated mites from results by Krogh (1995a), who added >1000 prey individuals weekly to each microcosm.

In the presence of dimethoate, food becomes even less available, mainly due to reduced prey reproduction, which can further affect predator reproduction or even survival. This may explain the significant decrease in numbers of juvenile *H. aculeifer* at the highest concentration tested (0.7 mg/kg) compared to the control in experiment 5A (Fig. 8). This decrease cannot be attributed to direct lethality due to the pesticide, since survival of juvenile mites was neither affected in a fresh nor in an aged soil contamination within the concentration range tested (experiment 2). Therefore, it is suggested that the inhibition of mite reproduction at 0.7 mg/kg in experiment 5A should possibly be attributed to food deficiency. This is supported by the fact that neither such inhibition was found by Krogh (1995a) nor in experiment 5B under similar experimental conditions and in the same concentration range (Table 3). In both cases, food deficiency was less serious than in experiment 5A, since Krogh (1995a) initially added 700–900 (instead of 100) springtails to the microcosms and prey reproduction at each test concentration was consistently higher in experiment 5B than in 5A. No reason was found for this difference in reproduction between experiments 5B and 5A. Although prey reproduction declined significantly with increasing dimethoate concentrations in experiment 5B, the higher basic level of reproduction probably prevented the microcosms from being completely depleted of juvenile prey, as was the case in experiment 5A.

The final number of juvenile mites is not directly affected by dimethoate exposure, but seems to depend on prey reproduction, which is affected by toxic stress and/or predation of the adults. This hypothesis is confirmed by a sensitivity analysis of a simulation model which is based on the same data set as that described in this paper (Axelsen *et al.* 1997). This sensitivity analysis indicates that the oviposition rate of *F. fimetaria* primarily affects the numbers of juvenile *H. aculeifer*.

Relevance of the Two-Species Toxicity Test System

The two-species toxicity test system described in this paper was developed to account for species–species relationships which may influence the response of an ecosystem to exposure to a toxicant.

The additional value of a multiple species test system is illustrated by the deduced hypothesis that predator populations are suppressed by a reduced availability of food, rather than by a direct effect of the pesticide. This secondary effect could not be derived from a single-species experiment, despite its crucial impact on the structure and functioning of an agricultural ecosystem after pesticide application. Shires (1985), who studied

the impact of cypermethrin and parathion-methyl on carabid beetles in a cereal crop, actually found a second reduction in carabid (predator) numbers after recovery of the population from the initial reduction caused by the pesticide application. This second decrease was attributed to a reduction of the food supply (mainly cereal aphids) rather than to a direct toxic effect of the compounds.

CONCLUSIONS

From the experiments described above, the following conclusions were drawn about the predator–prey relationships and the steering processes in a two-species toxicity test system:

1. Final numbers of adult *F. fimetaria* are affected by both predation and dimethoate exposure. Mites prey less efficiently on adult than on juvenile prey, because adult prey are able to escape from their killing grip.

2. At 0.357 mg/kg, the final number of juvenile *F. fimetaria* is mainly affected by predation on adults and juveniles. At 0.7 mg/kg, final numbers are also affected by dimethoate which reduces reproduction of *F. fimetaria*, either by mortality of the adults or by a reduced fecundity or fertility. Direct mortality of juvenile springtails caused by dimethoate exposure is less important.

3. Numbers of adult *H. aculeifer* are not affected after 21 days of dimethoate exposure at initial soil concentrations ≤ 0.7 mg/kg.

4. Reduced numbers of juvenile *H. aculeifer* at the highest dimethoate concentration of 0.7 mg/kg, should possibly be attributed to food depletion caused by a decreased availability of (juvenile) prey. At this concentration, direct mortality of juvenile mites is not important.

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