INFLUENCE OF FOOD LIMITATION ON THE EFFECTS OF FENVALERATE PULSE EXPOSURE ON THE LIFE HISTORY AND POPULATION GROWTH RATE OF DAPHNIA MAGNA

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Abstract—Laboratory ecotoxicity tests may not adequately evaluate the effects of pesticides, because they often do not include more environmentally relevant conditions, such as pulsed toxicant exposures and low food conditions. Therefore, we tested the effects of a pulse of the pyrethroid insecticide fenvalerate (FV) on the life history and population growth rate (r) of the cladoceran Daphnia magna. The daphnids were subjected to a 24-h pesticide pulse exposure (0.03, 0.1, 0.3, 0.6, 1.0, and 3.2 μg/L) under high and low food conditions and were monitored for 21 d. Chemical analysis showed that at t = 1 h, the nominal FV concentrations were reduced by 50 to 66%. Fenvalerate decreased survival and growth in the week following pulse exposure. Age at first reproduction increased, with consequent adverse effects on cumulative reproduction per living female and, therefore, on r. Thus, a short-term exposure of FV caused a long-term reduction on r as a result of increased mortality and a delay in development. Low food conditions exacerbated the effects of the FV exposure on juvenile survival and growth during the first week. This caused a much stronger reduction in r under low food conditions. We concluded that a pulsed FV exposure may result in long-term reduction of r that can be predicted only with more environmentally relevant toxicity tests, as described in the present study.

Keywords—Fenvalerate Short-term exposure Food limitation Recovery Population growth rate

INTRODUCTION

Laboratory ecotoxicity tests generally are performed under high-food conditions to obtain high control survival [1,2]. In the field, however, populations may experience periods of low nutritional supply [3–5] even in eutrophic waters when populations have grown to their carrying capacity [6]. Starved individuals have fewer resources available for physiological defense against stressors and, therefore, may be more sensitive to toxicants [7]. Indeed, several studies demonstrated an increase in toxicity of xenobiotics under limiting food conditions [8–11].

A second limitation is that organisms in laboratory ecotoxicity tests usually are exposed continuously to toxicants [12,13]. This may be in contrast to field populations that may be exposed in an episodic manner. Pesticide application in agriculture, for example, results in short-term pulse concentrations because of spray drift, drain flow, or edge-of-field runoff [14,15]. Hence, test designs with continuous exposure and no postexposure observation period may lack environmental realism, because they do not include either latent effects or recovery of the survivors once the pulse exposure has ceased.

The influence of low food conditions on short-term pesticide exposure has received little attention. Therefore, the aim of the present study was to evaluate the influence of food level on the effects of a 24-h pesticide exposure on the life-history characteristics and the instantaneous rate of population growth (r) of the model test species Daphnia magna. To this purpose, we performed life-table response experiments, which commonly are used in the assessment of potential risks of toxicants [16,17]. We were especially interested in examining the extent to which low-food conditions affected life-history characteristics and, ultimately, r in the period after the pesticide pulse exposure had ended. Because r integrates effects on juvenile and adult survival, reproduction, and age at first reproduction, this parameter has been proposed as a more relevant measure to predict consequences of toxicants on the population level [18].

The pyrethroid insecticide fenvalerate (FV) was chosen as the model pesticide. It is widely used for the protection of agricultural crops because of its high efficacy and generally low mammalian toxicity. Fenvalerate has been detected regularly in the aquatic environment [19], and peak concentrations of 0.1 to 6.2 μg/L have been measured in streams and estuaries [14,15,20]. A high sensitivity has, however, been found for nontarget organisms, such as aquatic macroinvertebrates [21]. Also, long-term effects in the structures of macroinvertebrate communities after a FV pulse exposure have been observed in experimental outdoor studies [22,23] and in the field [24].

MATERIALS AND METHODS

Daphnia magna culture

The experiments were performed with individuals of the cladoceran D. magna Straus (clone B; Bayer, Monheim, Germany). Continuous cultures of 10 adults/L in artificial Elendt M7 medium [25] were maintained at 20 ± 1°C under normal light (~1,400 lux) and a 16:8-h light:dark photoperiod. Other conditions were pH 7.4, conductivity of 630 μS/cm, and dissolved oxygen level of 7.15 mg/L. Once a week, a new culture
Effects of fenvalerate pulse exposure under food limitation

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was initiated using neonates (<24 h old) from a three-week-old culture. The newly released neonates were removed twice a week, and the medium was renewed three times a week. The daphnids were fed three times a week with a suspension of batch-cultured green algae (Desmodesmus subspicatus). Algae were cultured in algae medium according to the method described by Grimme and Boardmann [26] and was continuously aerated (3% CO₂). The algae were harvested during the exponential growth phase and centrifuged, and the pellet was resuspended in M7 medium. The food quantity, given as the equivalent daily ration, was 5.3 × 10⁶ cells/ml (carbon, 0.75 mg/L). Measurements were performed with a total organic carbon analyzer (Shimatzu TOC-5000 Analyzer, Duisburg, Germany) and a Coulter counter (Casy1 counter; Scharfe Systems, Reutlingen, Germany).

**FV exposure and measurement**

Fenvalerate, (RS)-α-cyano-3-phenoxybenzyl (RS)-2-(4-chlorophenyl)-3-methylbutyrate (CAS: 51630-58-1), was obtained from Riedel-de-Haën (high-performance liquid chromatography [HPLC] technical grade; purity, 99.9%; Seelze, Germany). Dimethylsulfoxide (DMSO) was used as a carrier solvent and obtained from Merck (HPLC technical grade; purity, 99.8%; Darmstadt, Germany). The maximum amount of DMSO in the test medium was 0.0003% (v/v). Our previous research did not show any detectable effect on D. magna at this DMSO concentration (unpublished results). Exposure consisted of seven different nominal concentrations for both food regimes (control, 0.03, 0.1, 0.3, 0.6, 1.0, and 3.2 µg/L). Actual exposure concentrations were determined in triplicate for both treatments at t = 1 h before food application. Absolute detection was limited to three concentrations (0.6, 1.0, and 3.2 µg/L). Additionally, a 1.0 µg/L test solution (n = 3) was measured at t = 24 h. Samples were measured by solid-phase extraction of 1-L volumes with C18 columns (Baker, Phillipsburg, NJ, USA), followed by gas chromatography–electron-capture detection (gas chromatograph: HP 5990, Series II; Hewlett-Packard, Avondale, PA, USA) and confirmed with gas chromatography–mass spectrometry (negative chemical ionization, Varian 3400 gas chromatograph; Varian, Walnut Creek, CA, USA) with a HP 7673 autosampler (Hewlett-Packard) that was directly capillary-coupled to the quadrupole mass spectrometer SSQ 700 (Finnigan, Bremen, Germany). Analytical measurements (mean ± standard deviation) of the 0.6, 1.0, and 3.2 µg/L test solutions at t = 1 h showed a reduction in the nominal concentrations of 50 to 66% (0.20 ± 0.00, 0.43 ± 0.12, and 1.47 ± 0.28 µg/L, respectively). The actual concentration of the 1.0 µg/L test solution decreased to 0.37 ± 0.06 µg/L after 24 h. Nominal concentrations are given in the following sections.

**Life-table response experiments**

The standard 21-d Daphnia reproduction test [25] was adjusted to study the influence of low food conditions on the effects of a FV pulse exposure on the life-history characteristics of D. magna. The life-table response experiments were carried out with two varying food levels: High, and low. A range-finding reproduction test with various food levels was conducted to establish the high food condition. The guidelines of the Organization for Economic Cooperation and Development provide a minimum cumulative reproduction criterion of 60 neonates per living female after 21 d. The high food condition used in the present study (equivalent daily ration, 5.3 × 10⁶ cells/ml; 0.75 mg carbon/L) resulted in 64 neonates per living female after 21 d. An approximately threefold-lower food level (equivalent daily ration, 1.5 × 10⁶ cells/ml; 0.21 mg carbon/L) was chosen as the low food condition that allowed females to produce 24 neonates after 21 d.

The life-table response experiments were initiated with newly released neonates (<24 h old) from a three-week-old culture and having a mean body length of 0.75 ± 0.05 mm (n = 40). Each treatment consisted of 20 individuals incubated in 20 glass beakers each containing 80 ml of test medium. The daphnids were exposed to FV for 24 h and were fed with the respective food levels of both experiments during the exposure to prevent food stress. The neonates were rinsed after 24 h and gently transferred into uncontaminated M7 medium. Feeding and renewing of the media was done three times a week. Other experimental conditions were similar to those described for the culture. On a daily basis, the following life-history characteristics of the daphnids were recorded: Survival (defined as swimming and immobilized animals), and the number of living neonates produced. Newly released offspring were removed. From these observed-daily life-history characteristics, age at first reproduction, mean brood number, mean brood size, and cumulative reproduction per living female at day 21 were determined. The r was calculated from the integration of the age-specific data on survival and fecundity probabilities. The r values were calculated iteratively from the Euler/Lotka equation [27,28]:

\[ r = \sum_{x=0}^{\Omega} l_m e^{-\alpha x} \]  

where \( r = \) per capita rate of increase for the population per day, \( x = \) age class (d; 1, 2, 3, . . . , \( \Omega \)), \( \Omega = \) oldest age class in the population (21 d in the present study), \( l_m = \) probability of surviving at age \( x \), \( m_x = \) neonates per mother at age \( x \), and \( e = 2.718 \).

Interactive calculations were performed to determine \( r \) values according to Equation 1. Uncertainties were estimated from jack-knife pseudovalues according to the method described by Meyer et al. [29].

To determine somatic growth of the daphnids during the test period, body lengths (distance from the middle of the eye to the base of the tail) were measured at days 7, 14, and 21. Measurements were performed using a Leica MS 5 microscope equipped with a Leica DFC 300 F Digital Camera and a Leica KL 1500 LCD light source (Leica Microsystems, Solms, Germany).

**Statistical analysis**

Data for all parameters were tested for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene’s test). When these conditions were not met, data were log(x + 1) transformed. Controls for both food levels were compared with each other using two-tailed t tests. Fenvalerate treatments within food levels were compared with the corresponding control using one-way analysis of variance (ANOVA; type 3 sums of squares), followed by Dunnett’s post-hoc test. To assess the contribution of FV concentration and food level to the observed variation, a two-way ANOVA was performed. For survival as a function of time, significant differences between FV treatments and the corresponding control were analyzed by conducting pairwise comparisons using Gehan–Wilcoxon survival analysis. A significance level of 0.05 was used for all
statistical tests, and three significance levels were adopted ($p < 0.001$, $p < 0.01$, and $p < 0.05$).

**RESULTS**

**Survival**

No mortality occurred in the controls during the experiments. The 24-h exposure to FV strongly impaired survival (Fig. 1). Mortality generally occurred between days 2 and 7 after the pulse exposure, and complete mortality at both food levels was observed at 3.2 $\mu$g/L. However, the lowest-observed-effect concentration (LOEC) for the low-food treatment was 0.6 $\mu$g/L, whereas for the high food treatment, the LOEC was 1.0 $\mu$g/L (Fig. 1). This indicated that low food conditions aggravated the effects of FV on survival of *D. magna*.

**Body length**

Low food conditions caused significantly ($p < 0.001$) smaller control body lengths at days 7, 14, and 21 compared to those observed under high food conditions (Fig. 2). Reduced growth after the short-term FV exposure was only apparent at day 7. However, the LOEC for the low food treatment was at 0.3 $\mu$g/L, whereas it was 1.0 $\mu$g/L for the high food treatment (Fig. 2). Two-way ANOVA demonstrated that at day 7, the statistical interaction term between the parameters FV and food was significant in explaining the observed variation in body length ($p < 0.05$). This indicated that the low food conditions significantly exacerbated the effect of FV on somatic growth of *D. magna* in the first week. Complete recovery of body length to the corresponding controls occurred at day 14 for both food levels, and no significant differences compared to the corresponding controls were observed.

**Reproduction**

Low food conditions significantly ($p < 0.001$) increased age at first reproduction (Fig. 3) and decreased mean brood number, mean brood size, and cumulative reproduction per living female at day 21 (Table 1) in comparison to the high food controls. Also, the 24-h FV exposure affected several of these reproductive traits.
Fenvalerate caused no significant reductions in mean brood size, in contrast to the low food treatment, which showed a significant increase at 1.0 \(\mu\)g/L (Table 1). The cumulative reproduction after 21 d was strongly impaired after FV exposure, although identical LOECs of 0.6 \(\mu\)g/L were exhibited for both food treatments (Fig. 3 and Table 1). Fenvalerate caused no significant reductions in mean brood size, in contrast to the low food treatment, which showed a significant increase at 1.0 \(\mu\)g/L (Table 1). The cumulative reproduction after 21 d was strongly impaired after FV exposure, although identical LOECs of 0.6 \(\mu\)g/L were exhibited for both food treatments (Fig. 3 and Table 1). Two-way ANOVA demonstrated no significant interaction between the parameters FV and food for reproductive output.

Population growth rate

The \(r\) in the control (Fig. 4) was significantly lower (\(p < 0.001\)) at the low food condition than at the high food condition. Fenvalerate pulse exposure strongly affected \(r\) as calculated for the 21-d posttreatment period. At day 21, the LOEC for the high food treatment was 0.6 \(\mu\)g/L, whereas the LOEC was 0.3 \(\mu\)g/L for the low food treatment (Fig. 4). Two-way ANOVA demonstrated that at day 21, the statistical interaction term between the parameters FV and food was significant in explaining the observed variation of \(r\) (\(p < 0.001\)). This indicated that the effects of FV on \(r\) were enhanced at the low-food condition.

### DISCUSSION

The present study revealed that a 24-h FV pulse exposure affected the \(r\) of *D. magna* during a subsequent 21-d period. The present study also showed that low food conditions exacerbated this long-term, adverse effect of the pesticide pulse.

The effects of FV on \(r\) can be traced back to impairments in the life-history characteristics of mortality, growth, and reproduction. Despite the brief FV exposure of only 24 h, FV-induced mortality still occurred up to day 7 and even resulted in complete mortality of the individuals at the highest test concentration. Similar long-term effects (>13 d) on survival after a 1-h exposure to the FV-isomer esfenvalerate have been demonstrated for juvenile *Gammarus pulex* [30]. A period with decreased growth (until day 7) caused by the FV exposure was followed by a period of rapid growth, leading to a uniform body length by day 14. Delayed development after FV exposure has been observed previously [23]. This likely results from either immobilization, leading to reduced filtration and assimilation rates [31], or changed resource allocation caused

### Table 1. Mean brood number (BN), mean brood size (BS), and cumulative reproduction per living female at day 21 (RPR) of *Daphnia magna* exposed to fenvalerate (FV) under high and low food conditions*

<table>
<thead>
<tr>
<th>Food regime</th>
<th>FV ((\mu)g/L)</th>
<th>BN</th>
<th>BS</th>
<th>RPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Control</td>
<td>4.86 ± 0.34</td>
<td>12.4 ± 0.6</td>
<td>64.5 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>4.70 ± 0.27</td>
<td>13.3 ± 0.6</td>
<td>61.7 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4.79 ± 0.27</td>
<td>12.6 ± 1.1</td>
<td>59.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>4.57 ± 0.30</td>
<td>12.6 ± 1.0</td>
<td>57.4 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4.33 ± 0.39*</td>
<td>12.2 ± 0.6</td>
<td>52.5 ± 5.2**</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.30 ± 0.41**</td>
<td>12.6 ± 1.5</td>
<td>53.7 ± 6.1*</td>
</tr>
<tr>
<td>Low</td>
<td>Control</td>
<td>4.26 ± 0.27</td>
<td>5.72 ± 0.27</td>
<td>23.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>4.15 ± 0.27</td>
<td>5.69 ± 0.35</td>
<td>23.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.89 ± 0.22</td>
<td>5.75 ± 0.32</td>
<td>22.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>3.69 ± 0.26**</td>
<td>5.78 ± 0.52</td>
<td>21.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>3.36 ± 0.34**</td>
<td>5.86 ± 0.41</td>
<td>19.6 ± 1.7*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.83 ± 0.43***</td>
<td>6.97 ± 0.70**</td>
<td>19.7 ± 3.1*</td>
</tr>
</tbody>
</table>

*Values are presented as the mean ± 95% confidence intervals. Asterisks denote significant difference compared to the corresponding control (analysis of variance; *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\)).*
by activation of detoxification systems. Effects of FV on mean brood size were not demonstrated, whereas reductions in the mean brood number and cumulative reproduction per living female were the result of the delay in age at first reproduction. Hence, the decrease in r because of the FV pulse exposure could be explained by delays in the individual life-history characteristics that were affected.

Population growth rate in the control treatment at the low food condition declined as a result of a delay in age at first reproduction, a reduced number of broods per time, a smaller mean brood size, and a lower cumulative reproduction per living female. The delay in age at first reproduction especially may have affected r, because population theory states that r depends mainly on the reproductive success of the younger individuals and that reproduction in older individuals is of less importance [32].

Low food conditions exacerbated the effects of short-term FV exposure on r. Several investigations have concluded that limiting food conditions increases the toxicity of xenobiotics, as summarized in the review by Heugens et al. [33]. A decreased survival of daphnids at low nutritional supply has been demonstrated earlier for continuous exposure to metals [34], pesticides [35], and metabolites of pesticides, such as 3,4-dichloroaniline [6]. The stronger reduction in growth under low food conditions matches well with the findings of studies regarding metals [9,36] but contradicts the observations of Barry et al. [35], who investigated the effects of several food concentrations on the toxicity of esfenvalerate to D. carinata. A plausible explanation for this discrepancy may be that Barry et al. compared two algae concentrations that differed only slightly. Smaller body lengths usually cause an increase in age at first reproduction [37]. However, this allometric relationship was, remarkably, not demonstrated in the present results, because no interaction between FV and food level was shown in the age at first reproduction. Apparently, daphnids were able to recover rapidly after day 7, as already indicated by the decrease one of the main outcomes of the present study (i.e., that recovery from FV pulse exposure is delayed by food-limiting conditions).

The incorporation of more environmentally relevant conditions in the present experiments indicated that low food conditions may aggravate the effects of a brief FV exposure on the r of D. magna even after 21 d, which was predominantly the result of an enhanced juvenile mortality. We also showed that tests with constant toxicant exposure may not adequately evaluate the effects of a FV pulse exposure on D. magna because of the persistence of lethal and sublethal effects after the cessation of exposure and the exhibited recovery trends in, for example, growth.

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