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Effect of an Acaricide on the Reproduction and Survival of *Daphnia magna*

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Cladocerans, especially *Daphnia* spp., are among the most favourable test animals in aquatic toxicology. The many advantages of daphnids, e.g., sensitivity to toxicants, parthenogenetic reproduction and the short reproductive cycle and life span, can hardly be found in combination in any other species (Bodar et al. 1988).

Chronic toxicity tests are used to assess long-term responses to contaminants. Although lethality may be used as an endpoint in chronic exposure tests, reproductive inhibition or growth retardation are generally considered more sensitive measurements, particularly for the estimation of sublethal responses. Effects of pollutants on the reproduction of *Daphnia magna* have been reported in several studies (Gersich and Milazzo 1988; Buhl et al. 1993; Ferrando et al. 1996a; Sanchez et al. 1998). In most cases reproduction was affected negatively by toxic agents. The intrinsic rate of natural increase (r) is a demographic parameter which expresses the growth potential of a population in an unlimited environment. Furthermore it incorporates information on survival and reproductive success of a population and has been used in ecological theory since 1930 (Barbour et al. 1989). Its use in toxicity testing is not unique (Day and Kaushik 1987; Ferrando et al. 1993); but it has not received a great amount of attention. It is a sensitive way of estimating toxicity to exposed populations in laboratory experiments because r integrates the measures of age-specific survival and fecundity, which increases the ecological relevance of the results (Van Leeuwen et al. 1985).

The main purpose of the present study was to assess the effects of low levels of the acaricide tetradifon, commonly used on many fruits and vegetables of the Valencia Community (Spain), on survival, reproduction and growth of *D. magna* using a standard 21-day static renewal procedure.

MATERIALS AND METHODS

The tetradifon (4-chlorophenyl 2,4,5-trichlorophenyl sulfone) used in these experiments was 99% pure as assayed by AFRASA Company (Spain). Stock solutions were prepared by dissolving the toxicant in acetone immediately prior to each experiment.

Daphnia magna organisms were obtained from continuous cultures maintained in our laboratory in 6L aquaria at 22±1°C, in dechlorinated tap water (total hardness, 240 mg/L as $CaCO_3$; pH 7.9±0.2; alkalinity, 4.1 mmol/L), 12hr:12hr 1ight:dark photoperiod and a density of below 50 animals/L. The medium was renewed two times each week and the daphnids were fed daily with the algae *Nannochloris oculata*. This algae was also continuously cultivated in our laboratory using a

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nutrient medium (Bischof and Bold 1983). Offspring were separated at regular intervals. Test animals were 6-24 hr juveniles, taken from cultures 3-5 weeks old.

Neonates (< 24 hr old) of *Daphnia magna* were exposed to four concentrations of tetradifon in a 21-days, static-renewal life cycle study. Fifteen 60 mL glass beakers, filled with 50 mL of test solution, were used at each of the four pesticide concentrations, plus the blank control and the acetone control (5 µL/L). An appropriate amount of acetone to each of the treatments was added in order to have the same concentration of solvent in all the test solutions. One cladoceran was randomly assigned to each of the beakers.

Water quality characteristics was constantly maintained by transferring the cladocerans to fresh test solutions or control water every day. The cladocerans were exposed to a wide-spectrum light intensity of 64 ft-c, with a 12:12 h 1ight:dark photoperiod. Approximately 5 x 10^5 cells/ml of *N. oculata* were added daily to each beaker to feed the daphnids. The young cladocerans used in this test were the second or a successive brood from stock animals (Meyerhoff et al. 1985).

Preliminary acute toxicity tests were conducted in order to calculate tetradifon LC50 data (Ferrando et al. 1996b). Based on these results, daphnids were exposed during 21 days to the following sublethal tetradifon concentrations: 0 (control), 0.10, 0.18, 0.22 and 0.44 mg/L, plus the acetone control. The size, fecundity and survival of the *D. magna* were monitored for each of the 15 replicates in this 21 d life cycle study . After 21 d, the length of each adult, from the apex of the helmet to the base of the tail spine, was measured to the nearest 0.01 mm.

The intrinsic rate of natural increase, r, was calculated using the formula of Lotka (1913): $\Sigma\ lx.mx.e^{-r.x} = 1$; where lx is the proportion of individuals surviving to age x, mx is the age-specific fecundity (number of females produced per surviving female at age x), and x in days. Since r calculated after 21 days is indistinguishable from r estimated for the entire life-span, due to the great importance of early reproduction (Van Leeuwen et al. 1985), all calculations were based on 21-day experiments.

Previous experiments carried out in our laboratory (Ferrando et al. 1996b) indicated that tetradifon concentration under the experimental conditions used was almost 90% of the original concentration after 24 hr. Based on these observations the test solutions were renewed every day in the experiments carried out with *D. magna.*

Data were analyzed using analysis of variance followed by Duncan test ($p<0.05$) with the SPSS computer program (Nie and Hull 1981). The maximum acceptable toxicant concentration (MATC) was also estimated. The MATC is defined as the estimated toxic threshold concentration falling between the highest concentration showing no effect (NOEC) and the next highest concentration showing a toxic effect (LOEC) when compared to the controls (Stephan et al. 1985).

RESULTS AND DISCUSSION

Tetradifon 24 hr LC50 value for *Daphnia magna* was previously calculated in our laboratory as 8.9 mg/L (Ferrando et al. 1996b). The influence of sublethal tetradifon concentrations on the survival of *D. magna* is shown in Table 1. Survivorship did not decrease significantly ($p>0.05$) with increasing concentration

Table 1. Size and fecundity of *D. magna* - F0 generation exposed to several concentrations of Tetradifon in a 21-d life study.

Tetradifon (mg/L)	Length (cm)	Longevity (days)	Days to first brood	No. of young per adult	Brood size	No. broods per adult
blank control	0.48±0.00	21.0±0.0	7.8±0.3	131.7±15.1	26.2±3.2	5.0±0.0
acetone control	0.48±0.01	21.0±0.0	7.6±0.3	127.8±4.0	25.9±0.9	4.9±0.1
0.10	0.46±0.01*	20.9±0.1	8.1±0.1	118.2±10.4	25.2±1.3	4.7±0.2
0.18	0.45±0.01*	20.8±0.1	8.7±0.2*	102.1±10.7*	22.3±2.5*	4.6±0.2*
0.22	0.45±0.00*	20.9±0.1	8.8±0.3*	97.3±5.6*	20.5±1.2*	4.7±0.3
0.44	0.38±0.00*	20.9±0.1	9.0±0.0*	65.9±2.1*	14.5±0.7*	4.5±0.3*

Values are means ± SD

* $p < 0.05$.

of tetradifon. At 0.44 mg/L concentration of the pesticide 99% of the daphnids were still alive.

The effects of tetradifon on the fecundity and size of *D. magna* after 21 d of exposure are summarised in Table 1. Reproduction was significantly ($p<0.05$) reduced at tetradifon concentrations higher than 0.18 mg/L, as a result of a decreasing number of broods and neonates per brood. Number of neonates born declined significantly ($p<0.05$) from 132 young (control) to 97.3 and 65.9 young at 0.22 and 0.44 mg/L, respectively. The onset of reproduction of *D. magna* was delayed at 0.18, 0.22 and 0.44 mg/L of tetradifon from 7.8 days in the control to 9.0 days at the highest tetradifon concentration. Populations of *D. magna* under control conditions and exposed to the acetone control had r values of 0.33 and 0.32 respectively (Fig. 1). A concentration of 0.1 mg/L tetradifon did not reduce r value, however at pesticide concentrations higher than 0.18 mg/L, r was reduced.

A significant reduction in mean caparace length of 21 day old daphnids was detected as tetradifon concentration increased to 0.10 mg/L or more (Table 1). This parameter decreased from 0.48 cm (controls) to 0.38 cm at 0.44 mg/L of the pesticide, respectively. Acetone controls did not differ significantly in any of the studied parameters.

The method used by USEPA (1979) for establishing the chronic value (MATC) from a *Daphnia* chronic study is total live young produced per animal over the course of the test (i.e., 21 days). If that method is used in this study, the no-effect tetradifon concentration (NOEC) will be 0.10 mg/L and the lowest-effect concentration (LOEC) 0.18 mg/L. Interpolation of the data indicates that MATC lies between 0.10 and 0.18 mg/L of the pesticide (geometric mean = 0.13 mg/L). The chronic data from this study can be used for formulating the application factor (AF) of tetradifon on *D. magna,* which has been used to estimate "safe" chronic concentrations of a chemical for a species. Dividing the chronic value generated by the present study (0.13 mg/L) by the daphnid acute LC50 value (8.9 mg/L) an AF of approximately 0.014 is obtained.

D. magna was a sensitive species to tetradifon. The sensitivity of this cladoceran to the pesticide is not surprising since crustaceans are closely related to other arthropods like insects or acaris. However, survival of *D. magna* was not reduced at concentrations higher than 0.44 mg/L of tetradifon. Several researchers (Day and Kaushik 1987) suggested that survival is only a good indicator when exposure to toxicants continues throughout the entire life-cycle of the organism. They found that survival of *Daphnia galeata mendotae* exposed to 0.01 µg/L of fenvalerate did not differ significantly from that of the controls for approximately 30-32 days. Similarly, other investigators have reported that reproduction was a more sensitive index of chronic pesticide toxicity to *D. magna* than survival (Buhl et al. 1993). In contrast, other investigators have found that survival and reproduction were both equally sensitive indicators of chronic toxicant stress (Gersich and Milazzo 1988, Ferrando et al. 1995).

In the present study, tetradifon produced a reduction in the reproduction capacity of *D. magna.* Similar results were reported by Day and Kaushik (1987) in *D. galeata mendotae* when exposed to 0.01 and 0.05 µg/L of fenvalerate. They observed a reduction in total young per female, mean brood size and number of broods. They did not find any effect in the number of days to first reproduction with the pesticide levels tested, whereas a significant increase ($p<0.05$) in the days to first reproduction was observed in the present study. A reduction in reproduction was

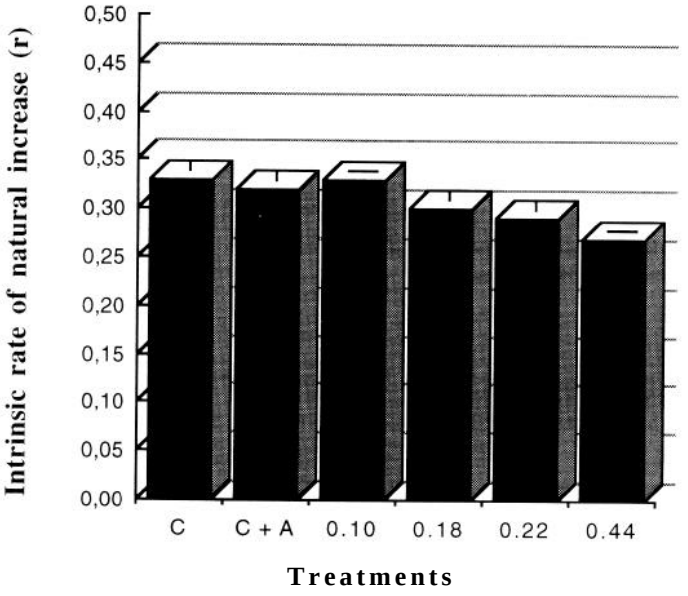

Figure 1. The effect of tetradifon on the intrinsic rate of natural increase (r) of *D. magna.* (*) $p<0.05$.

also found by Ferrando et al. (1996a) in *D. magna* when animals were exposed to the organophosphorus pesticide fenitrothion at a concentration of 0.011 µg/L and higher.

The intrinsic rate of natural increase (r) is found to be a sensitive parameter of toxicity due to the effect of tetradifon on reproduction. For populations such as daphnids with several overlapping generations, r will be determined primarly by the number of young per brood and the frequency of the first few broods will contribute relatively little to r. Tetradifon concentrations equal and/or greater than 0.18 mg/L significantly reduced the mean number of young in the first clutches resulting in a reduction in r. Daniels and Allan (1981) found that cohorts of *Daphnia pulex* exposed to increased concentrations of dieldrin showed little reduction in r until a concentration of 5 µg/L was reached. A reduction in the intrinsic rate (r) resulted as a consequence of chronic toxicant stress of fenvalerate (Day and Kaushik 1987) and diazinon (Sanchez et al. 1998) on *D. magna* .

A significant reduction in mean caparace length of 21 d old daphnids was detected at tetradifon levels equal and/or greater than 0.10 mg/L. Van Leewen et al. (1985) found that these effects occurred at levels comparables with their LC50 values when *D. magna* was exposed to the carbamates ziram and thiram. The length of *D. magna* was also reduced significantly after 21 d exposure to 0.25 and 0.27 ng/L of methylparathion (Fernández et al. 1995).
In another study, Ferrando et al. (1993) found that sublethal levels of certain pesticides such as lindane, dichloroaniline and PCP significantly decreased the rates of filtration and ingestion of the algae, *Nannochloris oculata* by the rotifer *Brachionus calyciflorus.* Similar results were reported by Villarroel et al. (1998)

studying the effect of pesticides on *D. magna*. Algal (*N. oculata*) feeding rates for *Daphnia magna* were significantly reduced upon 5 hr exposure to the pesticide tetradifon. Therefore, constant exposure of *Daphnia magna* to tetradifon over their entire life cycle may reduce their ability to obtain adequate nutrition. This could result in a reduction in the number of offspring produced, as indicated in the present study.

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