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Effects of Roundup® Herbicide on Populations of *Daphnia magna* in a Forest Pond

Lloyd D. Hildebrand, Druscilla S. Sullivan[1], and Thomas P. Sullivan
Northwest Ecological Animal Research Ltd., #1205-2233 Allison Road, Vancouver, B.C., Canada V6T 1K9

Roundup[R] herbicide[2] is a non-selective postemergence herbicide with great potential in agriculture and forestry. This herbicide will accelerate the transition from summer to autumn through defoliation which could alter the environment of streams and ponds in the treated area. A common inhabitant of such fresh-water ponds, as well as large lakes, are Cladocerans (MEGLITSCH 1972) such as Daphnia magna. A variety of reasons make D. magna and D. pulex among the most popular organisms for toxicity tests. Being fresh-water organisms, it is logical to use them in tests where the first environment to be contaminated is an aquatic one. TUNSTALL & SOLINAS (1977) have suggested that toxicity tests using Daphnia may be comparable to fish toxicity tests as well as have some financial and logistic advantages.

Daphnia are rather indiscriminate filter feeders and so would be exposed to toxicants and associated decomposition products that may be found on plants, bacteria, or in the soil. Daphnia could be adversely affected by any population or physiological changes occurring in their principal food source which is algae (MEGLITSCH 1972). In addition, D. magna is a particularly abundant invertebrate that forms a significant part of the diet of fish (CROSBY & TUCKER 1966). Any biomagnification or other adverse effect caused by a pesticide or its breakdown products entering an aquatic environment would ultimately affect fish.

As with toxicity studies in general, very few field experiments have been done with these organisms. Such studies are required to aid in predicting synergistic effects between the environment and the pesticide as well as any interspecific responses to disturbance.

The purpose of this study was to determine toxicological effects of a range of concentrations of Roundup herbicide on populations of D. magna in a field situation.

[1] Author to whom reprint requests should be sent.

[2] Roundup[R] herbicide by Monsanto, a commercial formulation containing glyphosate 356g/L present as isopropylamine salt

[R] Trademark of Monsanto Company

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METHODS AND MATERIALS

D. magna adults were obtained from the Bio-resource Engineering Department at the University of British Columbia on June 24, 1979. These specimens were transferred to a shallow plastic wading pool measuring 1.8 m in diameter and 0.3 m deep. The pool had been previously filled with pond water from the experimental site at the University of British Columbia Research Forest, Maple Ridge, B.C. and brought up to a temperature of $17^{\circ} \pm 2^{\circ}$ C.

The small population of 20-30 *D. magna* increased 100-fold within the next three weeks. Frequent observations showed that all organisms were females, and that gravid females contained 15-25 eggs each. These cladocerans were fed a daily ration of 1-3 bastersfull of liquified spinach, depending on the population size. This nutritious diet was made by thawing a package of frozen spinach leaves and blending this food with 500 ml pond water at high speed for 5 minutes. This diet produced a vigorously breeding, healthy population of animals. The population was maintained throughout the summer months with periodic harvesting and renewal of pond water to prevent overcrowding and fouling. Holding units, or pens, for the experiment, were constructed using rigid plastic strips and beige nylon stockings (see Figure 1). A rigid cylindrical plastic frame, 15 cm in diameter by 35 cm high, was constructed using plastic strips and PVC glue. Around this frame was stretched a single knee-high nylon. This nylon material was used because it has pore sizes which were large enough, when stretched, to readily permit diffusion of medium in and out of the pen and to sufficiently prevent clogging. The pores were also small enough that *D. magna* could not escape.

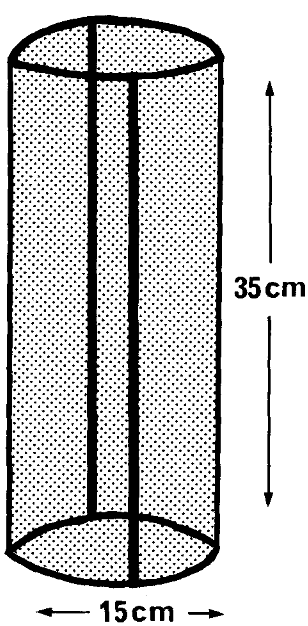

Figure 1. Holding unit or *Daphnia* pen constructed of plastic strips and nylon.

The experimental pond measured 60 by 24 m and reached a depth of 3-4 m. The pens were suspended in the pond using the fortuitous occurrence of small branches and other debris which emerged from the water surface. With some manipulation, all units were suspended in such a way that 30 cm of each pen was submerged. All units were placed in the pond for eight days prior to the experiment to test for any clogging of the nylon due to detritus or plant growth. Clogging in all cases was negligible.

Four stations were arranged in the pond such that overlapping effects between sites would be minimal. Three replicate pens were situated within a 1-m^2 area at each station. The four stations received the following Roundup herbicide treatments: control, field dose (2.2 kg/ha), 10x field dose and 100x field dose. The control station was closest to the incoming stream and the 100x dose station was located at the opposite end of the pond near the outgoing stream with the other two treatments in between.

On August 27, 1979, 12 samples of 25 neonate D. magna were taken from the breeding pool and transferred to the pens in the experimental pond. The animals were allowed a two-day period of acclimatization to the pond. On August 29, all sites (4 m^2 in area) were sprayed using a 500-ml garden plant mister. The control site was sprayed using pond water without Roundup herbicide. The remaining three sites were sprayed with their respective Roundup herbicide concentrations. Manoeuvring in the pond was done with minimal disturbance to the apparatus and organisms by using a small rubber raft.

Samples were taken from each station at 2, 4 and 8 days after exposure of D. magna to the herbicide. Sampling was done by carefully washing all contained matter in the unit to the bottom of the cylinder. The nylon was then cut, using scissors, about 5 cm from the bottom. This nylon, containing the retrievable D. magna was then thoroughly washed in a 250-ml wide-mouthed jar until clean. After washing, every part of the unit was carefully examined for any remaining organisms, dead or alive.

RESULTS AND DISCUSSION

Survival of the D. magna populations did not show any significant variation between control and experimental treatments at the three exposure times (Table 1). Due to the very slow circulation in the pond, it is safe to assume that all units were fully exposed to the herbicide for at least 2 hours. Water-logged pieces of wood placed in various areas of the pond did not move appreciably during that time.

The experimental design allowed for an effective exposure of D. magna to the toxicant. Passes were continually concentrated in the immediate vicinity of the pens during spray application. D. magna were not allowed access to depths greater than about 30 cm. In any toxicity assessment, extremely high doses of the herbicide or pesticide being studied (e.g. 100x field dose) must be tested along with field doses. This is necessary to predict responses of organisms to accidental, but inevitable, spills of the chemical directly into the environment.

TABLE 1

Survival rates (%) of <u>Daphnia magna</u>

Exposure	Control	Experimental Concentrations: Field Dose	10x Field Dose	100x Field Dose
2 days	92	100	96	92
4 days	100	100	100	100
8 days	92	100	data not available	96

The common situation is one in which an area containing a stream and/or pond protected by a vegetative canopy is sprayed with Roundup herbicide. Studies by SPRANKLE et al. (1975a and 1975b) and RUEPPEL et al. (1977) suggest that very little, if any, of the glyphosate or its decomposition products would reach the aquatic watershed via run-off. Glyphosate is initially inactivated in the soil by a process of reversible adsorption to clay and organic matter through the phosphonic acid moiety (SPRANKLE et al. 1975a and 1975b). Microorganisms in the soil rapidly degrade the glyphosate with minimal effect to themselves (RUEPPEL et al. 1977).

This experiment attempted to simulate an aerial application of Roundup herbicide to a forested area. Ponds and streams within an application site, which do not have an overhanging canopy of vegetation, could be susceptible to direct contact with the herbicide spray. The high survival rates of <u>D. magna</u> populations in this study (1x, 10x and 100x field dose) indicate that Roundup herbicide had no detectable effect on this particular invertebrate.

This study dealt with the possible immediate consequences of a direct hit of Roundup herbicide to a pond containing <u>D. magna</u>. Other studies were conducted at the same time concerning the effects of Roundup herbicide on diatom and testation populations (SULLIVAN et al. 1980). The results of these studies suggest that these organisms, which are a food source of <u>Daphnia</u>, are not adversely affected by this herbicide. Thus, delayed effects on <u>Daphnia</u> populations due to alterations in trophic levels on which these cladocerans depend should not occur.

Ecological studies on food webs indicate that effects on one trophic level, (e.g. primary consumers such as <u>Daphnia</u>) will ultimately affect another trophic level (e.g. secondary consumers such as fish which feed on the <u>Daphnia</u>). Such a food web system makes it economically important to study disturbances that may affect primary consumers such as <u>Daphnia</u> that are not themselves of direct commercial value. Another potential economic value in studying *Daphnia* populations in toxicological tests is the potential similarity between their tolerances and those of fish. Toxicology tests can be done on daphnids at 15-25% the cost of doing the same tests on fish (TUNSTALL & SOLINAS 1977). In addition, daphnids are generally more sensitive than rainbow trout to some toxic materials (LEDUC & ALI 1965). However, as with fish, the sensitivity of daphnids to certain toxic components can vary with both species type and locality (TUNSTALL & SOLINAS 1977).

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