Bull. Environ. Contam. Toxicol. (1991) 47:29–35

Bulletin of Environmental Contamination and Toxicology

Impact of an Organophosphate Herbicide (Glyphosate[R]) on Periphyton Communities Developed in Experimental Streams

A. P. Austin, G. E. Harris, and W. P. Lucey

Department of Biology, University of Victoria, Victoria, British Columbia, V8W 2Y2, Canada

The herbicide glyphosate (N-phosphonyl glycine; Roundup /Vision[R]) has been widely used and studied as an ecological management tool for terrestrial weed control. Few studies, however, have been conducted to determine glyphosate-induced effects in aquatic habitats, with the majority focused on toxicity to fish (especially salmonids; Wan et al. 1989; Mitchell et al. 1987; Servizi et al. 1987) and aquatic invertebrates (Folmar et al. 1979). Given the importance of primary producer and herbivore/carnivore trophic links, the almost complete absence of investigations into potential periphyton community deformation following glyphosate exposure (Goldsborough and Brown 1988) is surprising.

Recent laboratory work has shown that suppression of short-term photosynthetic rates can be induced in lentic periphyton subjected to glyphosate concentrations at 89 and 890 mg/L (Goldsborough and Brown 1988). In lotic systems sprayed with the herbicide, diatom community structural changes were hypothesized to have resulted from non-herbicidal environmental alterations (Sullivan et al. 1981). Similarily, post-application increases in periphyton standing crop were not attributed by Holtby and Baillie (1987) to the aerial spraying of glyphosate on the adjacent forest.

An alternative hypothesis which might explain the increased periphyton accrual noted by Sullivan et al (1981) and Holtby and Baillie (1987), and the latters' observation of post-application nutrient enhancement, is that glyphosate could potentially act as a phosphorus source (Goldsborough and Brown 1988). Thus, glyphosate could stimulate undesirable eutrophication of British Columbia's phosphorus limited, oligotrophic watersheds. Using low-level concentrations of glyphosate (1 - 300 ug/L) we investigated (i) herbicide toxicity and (ii) eutrophication effects on periphyton species composition and on biomass accrual in field-based, stream-troughs.

MATERIALS AND METHODS

The stream-troughs were located adjacent to Humpback Lake, a 12 ha (540 dam^3) surge reservoir, which receives its water from Sooke Lake (123°42'W, 48°33'N), the principal drinking water supply for Greater Victoria, British Columbia. Lying within the Coastal Mountain Limnological Region, the waters of these lakes are

Send reprint requests to Austin at the above address.

* Product of Monsanto Company, St. Louis, Missouri, USA.

oligotrophic and phosphorus limited (Lang and Austin 1984). Turbidity values are typically less than 5.0 (J.T. U.), with a mean annual Secchi Disc depth of 6 m. Typical winter (Dec - March) nutrient values include total organic N (0.08 mg/L), total Kjeldahl N (0.10 mg/L), total N (0.23 mg/L), N as NO_2 +NO_3 (0.14 mg/L), ortho P (<0.003 mg/L), total P (0.005 mg/L), total dissolved P (0.004 mg/L) and specific conductance (56 uS/cm); analysis after McQuarker (1976). Water temperature was 17.8°C (± 0.0, inter-trough variability) at experiment initiation and 5.9°C (± 0.0) at the end; intra-trough variability never exceed 0.1°C. Surface light intensity ranged from 0.08 to 1.24 uE/ cm^2sec; sub-surface values averaged 63% of surface intensities.

Stream-troughs (P.V.C.) had a rhomboid sectional profile [18.0 cm (base), open-top (26.0 cm) and sides (11.0 cm)], the angle of which (114°) minimized shading of contained periphyton; trough height was 10.0 cm, with a length of 245 cm. Paired streams received water, via a split manifold, from a header box supplying all troughs; the header-box was supplied with siphoned water, from constantly maintained depths of 0.5, 1.0 and 1.5 m; stream flows were 18.0 L/min. Periphyton was developed on 5.0 x 7.5 cm vertical borosilicate slides (racks of 10 parallel to waterflow), at 5 equal positions along the trough.

Experiment initiation began on August 26, 1989, and concluded November 21, 1989. The second over-lapping experiment commenced September 25 and was terminated December 21, 1989; the experimental protocols are outlined in Table 1. Nutrient enhancement consisted of di-sodium hydrogen orthophosphate (Na_2HPO_4) and sodium nitrate ($NaNO_3$) concentrates added (three day intervals) to 20.0L buckets, containing distilled water, to yield a continuous trough concentration of 0.003 mg/L P and 0.160 mg/L N (growth saturation values [Bothwell 1985; Ridley-Thomas 1990]). Added with nutrients, Vision® (glyphosate) was added to the buckets to yield final nominal concentrations of 0.0019, 0.1347 and 0.2874, and 0.0011, 0.0896 and 0.0977 mg/L in experiments 1 and 2, respectively. Glyphosate concentrations were estimated to be below detectable level (Wan et al 1989) or within typical post-application aquatic levels (Goldsborough and Beck 1989; Kreutzweiser et al 1989; Wan 1986).

Biomass accumulation, expressed as ash-free dry weight, was obtained from each trough by removing glass slides, scraping off the periphyton, and ashing by the method of Ridley-Thomas et al. (1989). Quantitative (numerical abundance (N.A.) and biovolume (Biov.)) and qualitative species compositions were determined from randomly selected slides, stored in a 2.0% buffered (di-sodium tetraborate) formalin solution. Periphyton growth was scraped from all slide surfaces; subsampled, Lugol's treated aliquots were settled in Utermohl (Zeiss) sedimentation chambers. Using a minimum of 5 random microscope fields and 500 algal units per sample, organisms were identified after Lucey et al. (1986). Biovolumes ($micrometers^3/cm^2$) were calculated (multiplying mean volume per species by numerical abundance) for each species using five geometric models (square, cylinder, sphere, ellipsoid or rectangle) which best approximated the organism's shape. Only the dominant species (>95% Biovolume) in each experiment have been graphed.

RESULTS AND DISCUSSION

A 2-wk period preceded measureable biomass in all troughs, for both experiments (Figure 1). In experiment 1, although the magnitude of biomass accrual differed between control troughs and those receiving glyphosate, all troughs exhibited

Table 1. Protocol for Experiment 1 (88 days) and Experiment 2 (87 days) indicating nutrient (N:Nitrate [$NaNO_3$]/P:Phosphate [Na_2HPO_4] and glyphosate (G) additions (+) to stream-troughs. Letters (a-j) refer to stream-trough treatments. Chem. = Chemical.

	Experiment 1					Experiment 2				
Chem.	a	c	d	e	b	f	h	i	j	g
N	-	+	+	+	+					
P	-	+	+	+	+					
G	-	+	+	+	-					
N						-	+	+	+	+
P						-	+	+	+*	+
G						-	+	+	+	-

* only nitrate added past this date.

biomass increase: decrease cycles. Except for the initial cycle, biomass in the control troughs was less than in those receiving glyphosate. The growth in control+nutrients recovered slowly prior to glyphosate addition following the first cycle, unlike troughs c-e (nutrients only). Before glyphosate addition, troughs b-e acted as replicates, demonstrating the low between-trough variablity. At all three glyphosate concentrations, post-glyphosate growth decreases were followed by biomass accrual values larger than in either control. An assessment of algal species composition (Figure 2) indicates all numerically dominant species were present in all troughs throughout the experiment; some patchiness was observed over time, between troughs and treatments. However, with the exception of N. acicularis, no species were lost following the addition of glyphosate. Numerically, diatoms were most abundant, with Achnanthes minutissima increasing to dominate all troughs, although representing only a minor proportion of biovolume. In laboratory stream studies, Steinman and McIntire (1986) observed that A. lanceolata increased over time, forming a dense understory, similar to that observed in our study. In our study algal biovolume was dominated by Chlorophytes and Synedra, Gomphonema and Tabellaria spp. Glyphosate did not appear to inhibit biomass accrual nor was it lethal to any of the pre-treatment dominant species, since all species were present throughout the experiment in all troughs. In troughs receiving the biocide, enhanced growth above control values suggested the phosphate constituent of this herbicide might be acting as a nutrient; previous work has demonstrated these oligotrophic waters to be phosphate limiting (Ridley-Thomas 1989). Although not quantified, in troughs c-e, there was a sudden loss of Simulum sp. larvae following glyphosate addition. If other herbivores were similarly affected by the herbicide, biomass increases may have occurred due to reduced grazing pressure. To further explore the possibility of glyphosate-enhanced eutrophication, a second experiment was conducted. Biomass accrual was enhanced by the addition of nutrients (Figure 1, g-j). As in the first experiment, pre-glyphosate accrual patterns in troughs g-j exhibited an increase-decrease cycle; however, these replicates showed little variation. Post-glyphosate biomass accrual patterns were similar in troughs g-j, again suggesting an algal response to nutrients rather than toxic effects. In the absence of a supplementary source of phosphorus at the time of biocide addition (trough j), biomass would be expected to decrease over time. However, the enhanaced biomass accrual in this trough supports the hypothesis that the biocide is acting as a nutrient source. In troughs g-i, in which phosphorus was added, biomass accrual was similar to j (Figure 1). In the control without nutrient

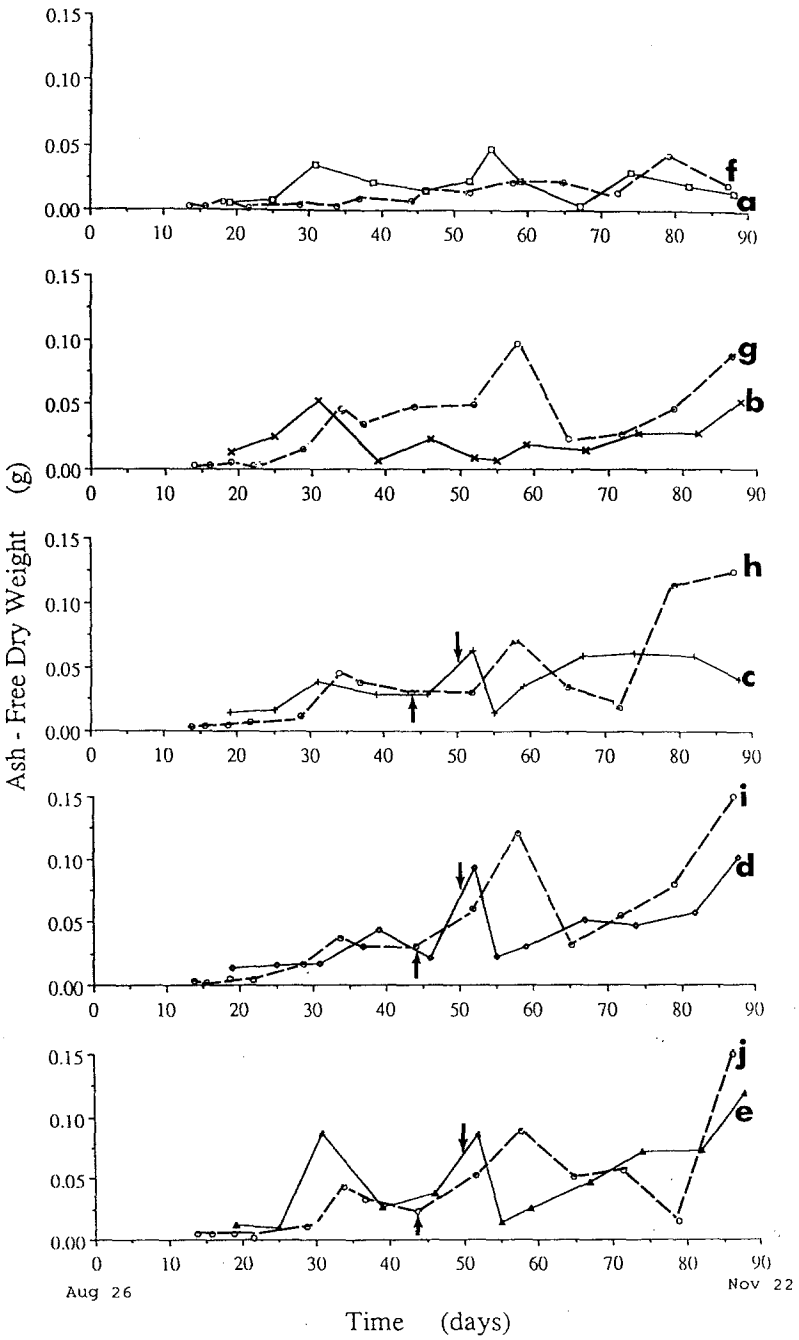

Figure 1. Accrued biomass in Experiment 1 (a-e) and 2 (f-j), developed in controls - lake water only (a,f); nutrients (b,g); and experimental - nutrients plus three nominal concentrations (mg/L) of glyphosate (0.0019-c, 0.1347-d, 0.2874-e), (0.0011-h, 0.0896-i, 0.0977-j); arrows indicate time of glyphosate addition.

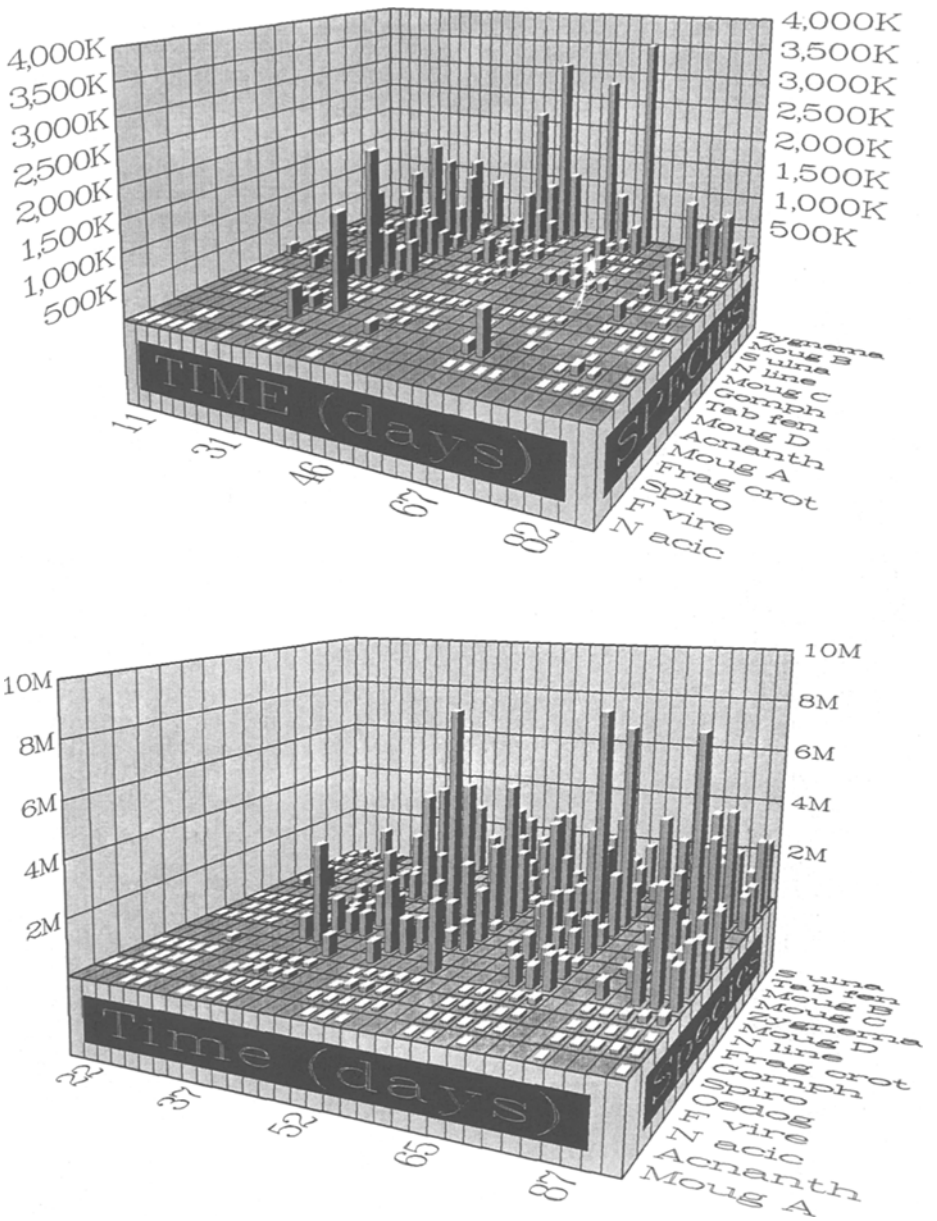

Figure 2. Biovolumes of dominant algal species in experiment 1 (upper) and 2 (lower), at five (blocked) sampling dates, within which treatments (exp. 1: a-e and exp. 2: f-j) are repeated from left to right; biovolumes (μ^3) on vertical axis. Species list - Achnanthes minutissima; Fragilaria crotonensis; F. viresens; Gomphonema acuminatum var. acuminatum; Mougetia spp.; Navicula acicularis; Nitzschia linearis; Oedogonium sp.; Spirogyra sp.; Synedra ulna; Tabellaria fenestrata; Zygnema sp.

additions (f), biomass remained relatively low. If biomass accrual was resulting from reduced herbivory, and if no phosphorus enhancement by glyphosate was occuring, then biomass values should have decreased in trough j once phosphorus was no longer added. The similar biomass accrual cycles between troughs h-i and g, suggests that herbivore effects are not significant since, hypothesizing that glyphosate eliminates herbivores, biomass increases should have occurred in troughs h-i, relative to that in g. As in experiment 1, no algal species were lost from the community after the addition of glyphosate. In particular, N. acicularis was present throughout the experiment, suggesting its post-glyphosate absence in the first trial was due to its low abundance and patchy distribution, and not to a toxicity response.

Diatoms were numerically dominant in all treatments and troughs, whilst biovolume was again dominated by Chlorophytes (4 of the top 6 species; Figure 2). Community dominance patterns, both numerical and volumetric, did not appear to exhibit a temporal change over the one-hundred twenty day period. The successional sequence in periphyton assemblages, seen in these experiments, are consistent with those identified by Steinman and McIntire (1986). Figure 2 indicates that adnate diatoms are early colonizors with stalked and chain-forming, vertically oriented, diatoms forming an initial canopy. Mature communities consist of a diatom understory and filamentous Chlorophyte canopy. In our study, 22 d after colonization, community architecture consisted of Synedra, Tabellaria, Fragillaria, and Achnanthes, a physiognomic pattern similar, but species richer, than that observed in laboratory stream studies (Steinman and McIntire 1986) . The addition of glyphosate, to a periphyton community, would thus appear to have little effect on subsequent successional patterns. The post-application increases in diatom standing crop, noted by Sullivan et al. (1981), may have also been the result of glyphosate induced eutrophication and not other environmental influences as suggested.

If the primary producer component of periphyton biofilm can use glyphosate or the surfactant as an alternate source of phosphorus, then, irrespective of other toxicological considerations, 'below detectable level' (Wan 1986) glyphosate-induced eutrophication of coastal oligotrophic waterways could indirectly affect salmonid habitat and other aquatic resource management concerns. The concentrations used in this study are within the range known to occur in lotic habitats following precipitation events or as a result of direct application (Wan 86). The paucity of published research on action of glyphosate on periphyton architecture, species composition, bioaccummulation, trophic transport (especially through secondary and tertiary consumer species preyed upon by juvenile salmonids) further recommends caution in the application of this herbicide.

Acknowledgments. We thank L. Taylor (Monsanto Co.) for supplying the herbicide; Science Council of B.C. (Grant #90 (RC-15)), N.S.E.R. Council of Canada (Grant A9537) and the Committee of Research and Travel (University of Victoria, B.C.) for funding; and Messr. K. Nickolichuk, P. Cobban, and J. Austin for technical support.

REFERENCES

Bothwell ML (1985) Phosphorus limitations of lotic periphyton growth rates: an intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). Limnol Oceanogr 30:527-542

Folmar LC, Sanders HO, Julian AM (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol 8:269-278
Goldsborough LG, Beck AE (1989) Rapid dissipation of glyphosate in small forest ponds. Arch Environ Contam Toxicol 18:537-544
Goldsborough LG, Brown DJ (1988) Effect of glyphosate (Roundup Formulation) on periphytic algal photo- synthesis. Bull Environ Contam Toxicol 41:253-260
Holtby LB, Baillie SG (1989) Effects of the herbicide ROUNDUP (glyphosate) on periphyton in Carnation Creek, British Columbia. Proceedings of the Carnation Creek Herbicide Workshop. March, 1989
Kreutzweiser DP, Kingsbury PD, Feng JC (1989) Drift response of stream invertebrates to aerial applications of glyphosate. Bull Environ Contam Toxicol 42:331-338
Lang S, Austin A (1984) Vertical distribution of biomass and species composition of a periphyton community on an artificial substrate in an oligo- trophic water-supply lake. Arch Hydrobiol 99(3):269-286
Lucey WP, Deniseger J, Austin AP (1986) A comparision of algal periphyton communities developed on arti- ficial substrata in two dissimilar containment systems. Naturaliste Can 113:153-165
McQuarker NR (1976) A Laboratory Manual for the Chemical Analyses of Waters, Wastewaters, Sediments, and Biological Tissues, 2nd Edition. Environment Laboratory, Ministry of Environment, Vancouver, British Columbia
Mitchell DG, Chapman PM, Long TJ (1987) Acute toxicity of Roundup and Rodeo herbicides to rainbow trout, chinook, and coho salmon. Bull Environ Contam Toxicol 39:1028-1035
Ridley-Thomas C (1989) Proximate composition of periphyton communities growing in experimental streams, M.Sc. thesis, University of Victoria, Victoria, B.C., Canada
Ridley-Thomas C, Austin AP, Lucey WP, Clark MJR (1989) Variability in the determination of ash free dry weight for periphyton communities: a call for a standard method. Wat Res 23(6):667-670
Servizi JA, Gordon RW, Martens DW (1987) Acute toxicity of Garlon 4 and Roundup herbicides to salmon, Daphnia and trout. Bull Environ Contam Toxicol 39:15-22
Steinman AD, McIntire (1986) Effects of current velo- city and light energy on the structure of periphyton assemblages in laboratory streams. J Phycol 22:352-361
Sullivan DS, Sullivan TP, Bisalputra T (1981) Effects of ROUNDUP herbicide on diatom populations in the aquatic environment of a coastal forest. Bull Environ Contam Toxicol 26:91-96
Wan MT (1986) The persistence of glyphosate and its metabolite amino-methyl-phosphonic acid in some coastal British Columbia streams. Dept of the Environment, EPS, Pacific and Yukon Region, Report 85-01
Wan MT, Watts RG, Moul DJ (1989) Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. Bull Environ Contam Toxicol 43:378-385
Received November 13, 1990; accepted December 27, 1990.