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Effect of Glyphosate (Roundup® Formulation) on Periphytic Algal Photosynthesis

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Glyphosate (N-(phosphonomethyl)glycine) is a non-selective, broad spectrum, post-emergent herbicide recognized for use in the control of grasses, sedges and broad-leaved weeds (WSSA 1983). The Roundup(R) commercial formulation[1] is widely used in agricultural and silvicultural practise in North America and elsewhere.

Glyphosate may enter aquatic systems through off-site movement from treated terrestrial land in herbicide spray drift, in surface runoff (Edwards *et al.* 1980) or adsorbed to suspended particulate matter (Bowmer 1982). Glyphosate has also been evaluated for use as an aquatic herbicide (Seddon 1981) and is registered in the United States as Rodeo(R)[1] for this purpose.

There have been several studies of the toxicological effects of glyphosate on aquatic plants grown in laboratory culture (*e.g.*, Hartman and Martin 1985) but few studies involving natural *in situ* plant communities. Sullivan *et al.* (1981) studied effects of a 2.2 L/ha Roundup application on the density of some periphytic diatoms, but could not differentiate phytotoxicity from natural seasonal succession. The objective of the present study was to assess the effects of the Roundup formulation of glyphosate on short term carbon assimilation by periphytic algal communities collected from six small forest ponds.

MATERIALS AND METHODS

The study ponds are located in boreal forest on the Canadian Precambrian Shield bordering Lake Winnipeg, Manitoba (96° 10'W, 50° 37'N). The pond basins (surface areas 0.2-0.7 ha) were excavated circa 1959 during

[1]Products of Monsanto Company, St.Louis, Missouri, USA.

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construction of an adjacent highway, and have since filled with water (mean depths 0.9-1.5m), become surrounded by woody and herbaceous vegetation, and colonized by a diverse nonvascular and vascular flora.

The ponds are within an area subject to silvicultural glyphosate use although the study sites had not been treated at the time of this study. Background levels of glyphosate and its primary metabolite aminomethylphosphonic acid (AMPA) in pond waters were below a 0.50 *ug*/L detection limit.

Three ponds (Raspberry, Pine, and Birch) were dystrophic as a result of drainage from surrounding wetlands (Secchi depth 0.43-2.00 m, true color 35->50 Pt units) while three ponds (Spruce, Golden, and Hike) were comparatively less colored (Secchi depth 1.35->3.18 m, color <5-20 Pt units). All waters were relatively dilute (specific conductance <500 *uS*/cm), although concentrations of major cations (Na, Ca, Mg) and total carbonate alkalinity were consistently higher in Spruce Pond than in all others (Goldsborough & Brown 1986).

Extruded acrylic rods 30 cm in length (Goldsborough *et al.* 1986) were used as artificial substrata for periphytic algal colonization. Ten substrata were imbedded vertically in a flat concrete base, which was laid on the sediment surface in water of about 45 cm depth. The substrata were scored to facilitate collection of four 2.5-cm segments in the 10-20 cm interval on each rod (height above base), and a 10-cm segment from the 20-30 cm interval. Substrata were placed in each pond in late summer and allowed to colonize with periphyton for 44-52 days (Table 1). Two sets of substrata were positioned at one site in Birch Pond to provide algal material for a preliminary experiment to determine the appropriate range of herbicide concentrations for further testing.

Thirty 2.5-cm segments were sampled from 10 rods (3 of 4 segments / rod) from each pond and placed into clear glass tubes containing 20 mL filtered (Whatman GF/C) pondwater. The remaining upper 10-cm segment was placed into an empty tube for chlorophyll analysis. All tubes were transported to the laboratory and allowed to reach the incubation temperature (16^{o}C) before commencement of herbicide and radioisotope additions. Samples for chlorophyll analyses were immediately frozen. Serial aqueous dilutions of Roundup (35.6% glyphosate acid formulated as an isopropylamine salt) were prepared such that 0.1 mL aliquots added to the incubation media yielded glyphosate concentrations of 8.9×10^{-6} to

8.9×10^{-2} mg/L (5.3×10^{-11} to 5.3×10^{-3} M). Herbicide treatments and one distilled water control treatment were assigned to samples according to a randomized incomplete block design with three replicates per treatment level. One half milliliter of $NaH^{14}CO_3$ (18.5 kBq/mL) was added to each tube. All samples were incubated in a controlled environment chamber at a light intensity of 200 umoles/m^2/s for 4 h with intermittent agitation. Substratum segments and dislodged periphyton were then collected onto 0.45 um filters under vacuum and postwashed with 10 mL distilled water. The samples were fumed over concentrated HCl to liberate residual inorganic ^{14}C, and placed in vials containing 5 mL Scintiverse (Fisher Scientific Ltd., Edmonton, Canada) cocktail. Radioactivity was determined via scintillation counting. Total inorganic carbon in the incubation media was estimated from titratable alkalinity, pH and incubation temperature, and used to calculate carbon fixation rates (ug C/cm^2/h) of periphyton samples (Goldsborough & Robinson 1983).

Photosynthetic inhibition (I) was calculated as follows:

$$I = 100 \times (R_C - R_S)/R_C$$

where R_S and R_C were the radioactivities of glyphosate-treated and corresponding control treatments respectively. These data and $\log_{10}$-transformed herbicide levels were fitted to the probit function using the BASIC computer program of Trevors and Lusty (1985). A chi-squared goodness of fit test was used to test a null hypothesis that the fitted line adequately modelled the data (using a critical chi-squared value at 6 d.f. and 95% confidence of 12.59), after which EC_{50} (glyphosate level resulting in 50% inhibition of carbon fixation) and 95% confidence limits were calculated (Trevors and Lusty 1985).

Chlorophyll was eluted directly from thawed substratum segments in 10 mL neutralized 90% methanol for 24 h in the dark at room temperature. The concentration of chlorophyll *a* in the extracts was determined after the method of Lorenzon (1967).

RESULTS AND DISCUSSION

There were clear differences in the algal colonization of substrata from each pond (Table 1). On bases of total chlorophyll content and absolute inorganic carbon fixation rates, Pine Pond substratum samples were most heavily colonized, while Spruce Pond samples were

least. Algal biomass on substrata from Raspberry, Golden and Hike Ponds, and preliminary samples from Birch Pond were similar, while the second series of samples from Birch Pond supported less biomass than the first (Table 1).

Table 1. Estimated biomass (mean ± SD, n=10) of periphytic algae on artificial substrata

Pond	Colonization time (d)	Chlorophyll *a* (ug/cm^2)	Carbon fixation rate ($ug/cm^2/h$)
Spruce	46	0.02 ± 0.01	0.15 ± 0.10
Raspberry	46	0.19 ± 0.04	0.68 ± 0.13
Pine	49	0.26 ± 0.05	1.12 ± 0.14
Golden	49	0.14 ± 0.04	0.45 ± 0.17
Hike	52	0.14 ± 0.07	0.68 ± 0.21
Birch expt.1	44	0.14 ± 0.02	0.88 ± 0.29
expt.2	52	0.07 ± 0.04	0.70 ± 0.18

The short term photosynthetic rates of intact periphyton communities were significantly impacted by glyphosate additions (Figure 1). Carbon fixation rates of periphyton samples from Birch Pond treated with 8.9 $x10^{-6}$ to 8.9 mg/L glyphosate were similar to those of control samples. Rates were 20±4% (mean ± SD, n=3) and 14±3% of the control at 89 mg/L and 890 mg/L respectively. It seems reasonable to infer that glyphosate concentrations less than 0.89 mg/L had no effect on short term algal photosynthesis, while the EC_{50} value lies between 8.9 and 89 mg/L.

Based on the preliminary experiment, herbicide treatments of less than 0.89 mg/L were excluded from further experiments. The glyphosate concentrations used for Spruce and Raspberry Pond bioassays were 0.89, 8.9, 89, 180, 360, 530, 710, 890 and 1800 mg/L glyphosate. The concentrations used in all other bioassays were 0.89, 8.9, 18, 36, 53, 71, 89, 890 and 1800 mg/L.

Except for Spruce Pond data (Figure 2A), there was general agreement in the level of toxicity found for algal communities from each of the study ponds (Figures 2B-F). A threshold glyphosate concentration, below which photosynthesis was not sigificantly different from untreated control samples, was approximately 0.89 mg/L in Pine (Figure 2C), Hike (Figure 2E) and Birch (Figure 2F) Ponds, 8.9 mg/L in Raspberry Pond (Figure and 18 mg/L in Golden Pond (Figure 2D). Between 8.9 and 1800 mg/L, photosynthetic activity decreased with increasing herbicide concentration in most ponds. Notably, carbon fixation was not completely inhibited at

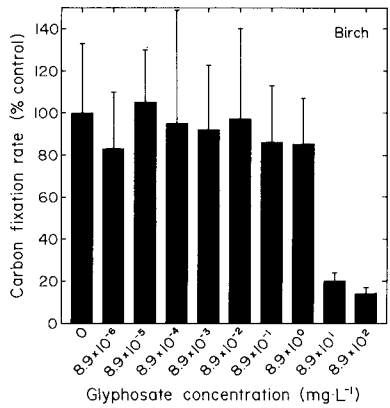

Figure 1: Response of 4-h periphytic algal carbon fixation rate (% unmanipulated control ± SD, n=3) to glyphosate (Roundup) additions.

the highest glyphosate concentration used in these experiments (1800 mg/L) where rates amounting to 11-27% of the control were observed.

Due to the clear departure of Spruce Pond samples from a typical dose-response curve, the probit line fitted to those data was not significant at the 5% level (chi-squared = 93.26). An EC_{50} value therefore could not be calculated. The lack of significance of the fitted probit lines for Raspberry Pond (chi-squared = 48.28) and Golden Pond (chi-squared = 255.86) was due to a lack of treatments between 8.9 and 89 mg/L, and high replicate variability, respectively. However, visual inspection of these data indicates that EC_{50} values would lie between about 8.9 and 89 mg/L glyphosate for both ponds (Figure 2B,D). Slight apparent stimulation of photosynthesis occurred at 8.9 mg/L in Golden Pond and at 0.89 mg/L in Raspberry Pond, although no explanation can be offered here.

Calculated EC_{50} values were statistically significant in Pine Pond (EC_{50} = 69.7 mg/L; 95% confidence limits = 50.7, 98.5; chi-squared = 2.15), Hike Pond (EC_{50} = 44.4 mg/L; 95% C.L. = 33.4, 60.5; chi-squared = 12.58) and Birch Pond (EC_{50} = 35.4 mg/L; 95% C.L. = 29.2, 43.5; chi-squared = 11.50). Overlapping 95% confidence intervals point to differences between the three estimates but do not represent valid comparisons of interpond variability. Despite this, there is no inherent reason why EC_{50} values should be the same for samples from each of these ponds because intact periphyton commun-

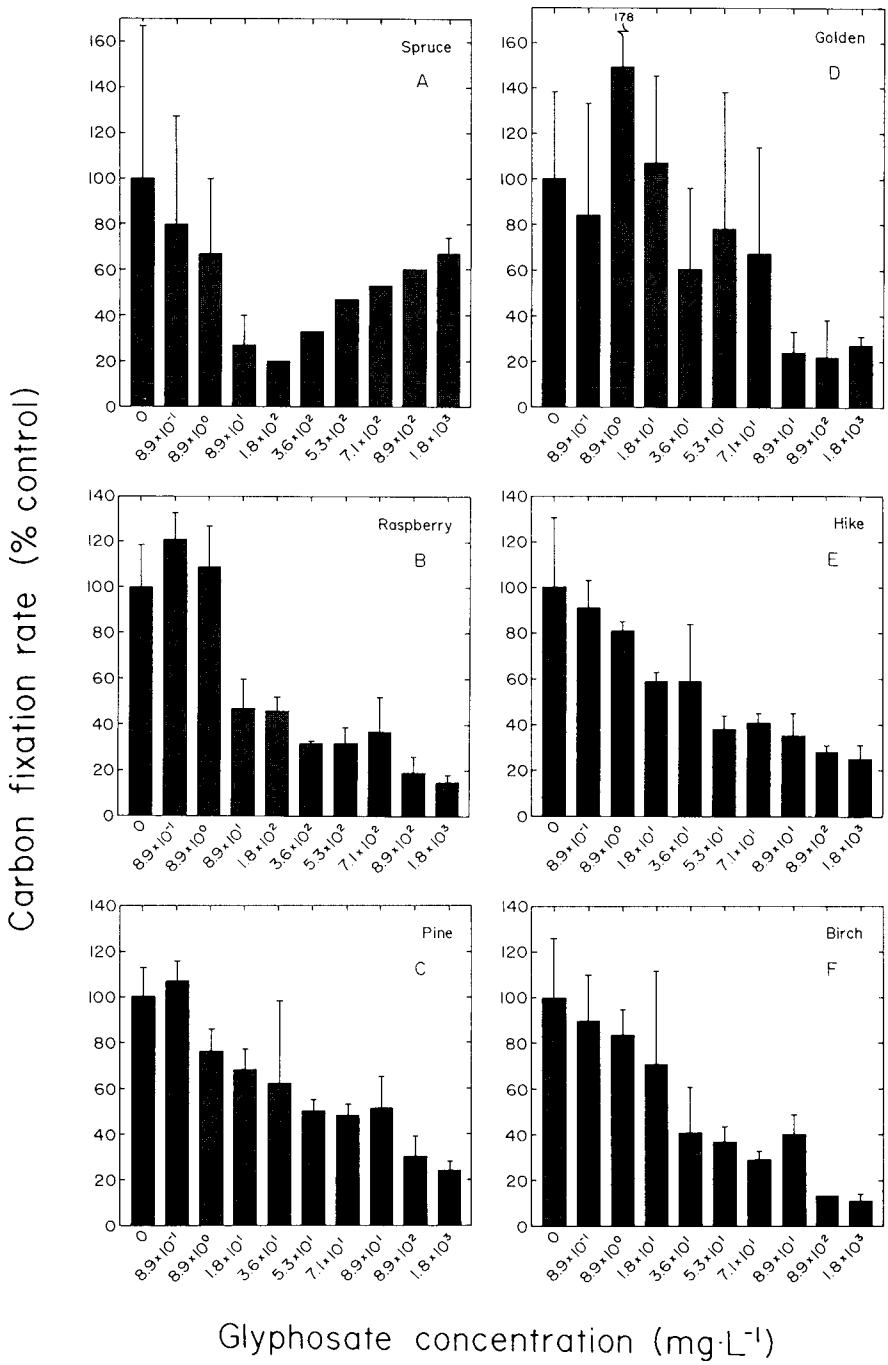

Figure 2: Response of 4-h periphytic algal carbon fixation rate (% unmanipulated control ± SD, n=3) to glyphosate additions. Note the varying units on the abscissa for each panel.

ities consist of a complex assemblage of organisms differing in sensitivity to glyphosate.

Van Rensen (1974) observed a 50% reduction in oxygen evolution by a culture of the green alga *Scenedesmus* sp. after 60-90 min exposure to $7x10^{-4}M$ (118 mg/L) glyphosate. He concluded from further studies of DCPIP reduction in spinach chloroplasts that glyphosate directly inhibits photosynthetic electron transport in photosystem II. However, this was disputed (Richard *et al.* 1979) as a consequence of concomitant pH change in weakly buffered media. Glyphosate is known to be a weak acid and can release hydrogen ions (Sprankle *et al.* 1975a). If glyphosate, or a component of the Roundup formulation, directly inhibits photosynthesis in periphytic algae, then it is clearly less effective than other known photosynthetic inhibitors. For example, an EC_{50} for the triazine herbicide simazine to periphyton photosynthesis was about 0.8 mg/L (Goldsborough and Robinson 1988).

Factors which may bear on observed glyphosate toxicity but which were not considered here include transport limitation in thick periphyton films, interactions of formulation constituents with pondwater chemistry, herbicide inactivation through adsorption to suspended solids and substrata, and sequestering within cells, or metabolism of the herbicide by periphytic organisms as a phosphorus source. Data describing the relative significance of these factors will be necessary to fully evaluate potential *in situ* phytotoxic effects of Roundup. Finally, the present findings relate only to responses of algal photosynthesis to glyphosate during a 4-h exposure period and may differ from longer term growth effects due to interference with various aspects of cell metabolism. Field evaluation of long term responses of algal productivity and biomass to glyphosate additions are currently underway at the study area.

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