buried in the interior of protein molecules.

Further studies are needed to elucidate more closely the factors responsible for the improvement of gelling properties of DEW proteins when heated in the dry state.

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Persistence, Movement, and Degradation of Glyphosate in Selected Canadian Boreal Forest Soils

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Persistence, mobility, and degradation studies of glyphosate, *N*-(phosphonomethyl)glycine, under actual field conditions of boreal forest soils of Ontario, were undertaken after spraying Roundup at the rate of 2 kg of active ingredient (AI)/ha. Soils at three depths were collected and analyzed for residues of glyphosate and its metabolite (aminomethyl)phosphonic acid. Glyphosate was found to remain consistently to a level below 50% of the highest residue values observed beyond 24 days. More than 95% of the total herbicide residue was found in the upper organic layer at any time. There was no evidence of lateral movement of the glyphosate either in runoff water or through subsurface flow. In general, concentrations of the metabolite (aminomethyl)phosphonic acid were very low.

The herbicide glyphosate (GLYPH), *N*-(phosphonomethyl)glycine (Monsanto's Roundup), is an environmentally safe broad-spectrum herbicide having a potential for use in silvicultural programs such as site preparation, conifer release, and nursery stock production. This herbicide has been recommended for use in agricultural as well as forestry substrates in Ontario. Studies on the behavior of glyphosate in or on soil have been reported (Torstensson, 1982; Stark, 1982; Salazar and Appleby, 1982; Torstensson and Stark, 1979, 1981; Rueppel et al., 1977; Torstensson and Aamisepp, 1977; Sprankle et al., 1975a,b). However, inadequate data exist as to its behavior under boreal forest conditions in Ontario, and hence this study was undertaken.

EXPERIMENTAL SECTION

Reagents. Glyphosate (98%) and (aminomethyl)-phosphonic acid (AMPA) (94%) were supplied by Monsanto Chemical Co. Trifluoroacetic anhydride and trifluoroethanol were purchased from Aldrich Chemical Co. Anhydrous sodium sulfate was heated at 140 °C overnight prior to use. All organic solvents used were pesticide grade (Caledon Laboratories, Georgetown, Ontario, Canada).

Location and Experimental Design. One sand site for persistence and leaching studies and one clay site for mobility study were selected. The sand site was part of a recently planted jack pine plantation that also contained the occasional blueberry plants. The clay site was in an open cutover covered by weeds and the occasional remnant of the original forest, namely white birch, black spruce, and poplar. The sand and clay sites were located in Harker (48°30′ N, 79° W) and Lamplugh (48°35′ N, 79° W) townships, respectively, about 40 km east of Matheson in the district of Cochrane, Ontario. Each site (20 m × 20 m) was divided into five replicate strips separated by buffer zones (1 m × 20 m). Each strip (2 m × 20 m) was further subdivided into 10 squares (2 m × 2 m) as sampling plots.

Site Preparation. All dead wood, live brush, and as much vegetation as possible were manually removed from the site with minimal disturbance of the duff layer (5–10 cm in depth). For the mobility study, dead wood and other

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Table I. Characteristics of the Study Soils[a]

	soil[c] fraction	clay[b] mobility site	sand[b] persistence site
pH	O	4.48	3.50
	M	4.43	3.73
% clay	O	0.00	0.00
	M	87.60	5.60
% silt	O	0.00	0.00
	M	12.40	14.00
% sand	O	0.00	0.00
	M	0.00	80.40
% organic matter	O	29.47	39.70
	M	0.00	0.76
CEC, mequiv/100 g	O	21.40	18.50
	M	3.80	5.30
% moisture content	O	8.26	7.71
	M	0.85	0.56
field capacity	O	137.43	24.47
	M	38.94	9.24

[a] Soil classification (Jotcham, 1985): clay of the Ryland series, Orthic Humic Gleysol type; sand of the Abitibi series, Orthic Humo-Ferric Podzol type. [b] Soil texture. [c] Key: O = organic (5–10 cm); M = mineral (20–25 cm in depth of the total 30-cm soil core).

Table II. Chemical Application to the Sand Site

	persistence site		
rep	vol appl, mL	rate appl, kg/ha	rate appl from deposit sheets,[a] kg/ha
A	1360	2.550	1.734
B	1230	2.300	1.595
C	1100	2.060	1.607
D	1120	2.100	1.445
E	955	1.790	1.362
av	1153	2.160	1.549

[a] An average of the three replicate deposit sheets per application zone.

Table III. Chemical Application to the Clay Site

rep	spray strip
vol appl, mL	1430
rate appl, kg/ha	2.680
rate appl from deposit sheets,[a] kg/ha	1.789

[a] An average of three replicate deposit sheets.

matter thought to have a potential for runoff channeling were removed from the site, and an application strip at the top of the slope was cleared as above. A back-hoe was used to prepare a trench at the bottom of the slope for the collection of the runoff water.

Soil. The characteristics of the typical boreal forest soil of Ontario are given in Table I.

Chemical Application. Chemical application were made on June 20, 1984, to the zone of application for the mobility site and on July 19, 1984, to the replicate strips in the persistence site. Glyphosate, *N*-(phosphonomethyl)glycine, was applied as an aqueous solution of Roundup (35.6% AI) with a pestex backpack sprayer (boom length 2 m, number of nozzles 4, nozzle type TEE Jet AL 8004) using compressed air (200 kPa) as a propellent. An application rate of 2 kg (AI)/ha was targetted, and actual rates were determined by the use of deposit sheets as well as by the reservoir volumes before and after spraying (Tables II and III). Deposit sheets prepared from 20 cm × 20 cm glass plates wrapped in aluminum foil were placed in each application strip. Immediately after application the foil sheets were unwrapped (thereby quantitatively trapping the deposit), labeled, and frozen until analyzed.

Sampling. Soil cores were taken from sampling plots with use of a random number table. Samples were also collected at 3, 6, 9, and 12 m downslope from the chemical application zone in case of mobility site. A soil auger (length 54 cm, diameter 10 cm) was driven to the depth of 32 cm with a sledge hammer. The bottom 2 cm of the core was discarded and the adjacent 15 cm mineral soil (M2, 15–30 cm) collected. The remainder of the core was divided into organic and mineral (M1, organic to 15 cm) layers and collected separately. The sections were bagged, weighed, and stored at −20 °C. The sampling schedule was 0, 2, 7, 14, 28, 43, 78, 125, 365, 721, and 792 days and 0, 1, 6, 14, 49, 96, 335, 691, and 762 days for the mobility and persistence sites, respectively. Water samples of 1 L were collected from the trench and stored at −20 °C.

Weather. Field weather stations were used to monitor rainfall and temperature. On examination of the rainfall data produced from these, it was discovered that they had intermittently malfunctioned and as a result these data were discarded. However, weather data were obtained from the Ministry of Natural Resources (MNR) district weather station in Kirkland Lake, Ontario, which is approximately 40 km southwest from the study sites. It was felt that these data from the MNR are the second best source, which will provide a reasonable approximation of the climatic conditions during the experimental period. These weather data were used to indicate that the year 1984 was climatically normal compared to those in past years (Table IV).

Soil Preparation. Frozen soil cores were allowed to thaw at 25 °C, air-dried, homogenized in a heavy-duty stainless steel blender, and sieved through a 10-mm-mesh brass sieve (Feng and Klassen, 1986). The final moisture content was 5–7%.

Extraction and Cleanup. An analytical methodology has been established by our laboratory for the isolation and quantification of glyphosate and its metabolite AMPA and has been reported earlier (Roy and Konar, 1988). To a subsample (5 g) of the finely ground, homogenized soil in a 250-mL screw-cap bottle was added concentrated phosphoric acid (0.5 mL). The bottle was capped and shaken manually for 2 min. Deionized water (100 mL) was

Table IV. Monthly Rainfall and Temperature Data for the Period 1975 (May–September) to 1984 (May–September)

month	1984	1983	1982	1981	1980	1979	1978	1977	1976	1975
					Rainfall, mm					
May	54.8	118.4	46.5	46.6	41.0	80.5	55.9	19.9	69.4	101.6
June	128.5	70.4	69.5	94.1	49.4	151.0	143.2	118.2	71.3	83.9
July	93.7	55.2	71.7	46.3	44.8	96.4	141.8	54.1	95.8	49.0
Aug	88.5	64.0	68.5	64.2	79.8	94.1	103.0	71.9	40.2	50.3
Sept	41.0	38.3	85.0	79.0	111.2	92.0	62.1	58.8	156.4	103.9
					Temperature, °C					
May	10.1	10.1	17.7	12.1	14.9	14.4	19.6	17.0	12.1	17.6
June	17.4	19.3	16.0	18.0	16.1	19.0	16.6	16.9	22.2	16.1
July	21.0	21.5	20.9	23.4	21.7	21.6	20.2	21.1	20.2	22.0
Aug	20.4	20.9	15.0	21.0	20.9	17.3	19.0	17.3	20.5	20.2
Sept	13.6	15.9	12.4	11.7	11.8	13.7	12.6	13.7	12.8	11.0

added followed by the addition of chloroform (50 mL) and the resulting slurry quantitatively transferred to a small domestic blender. After blending for 2 min, the solution was filtered under suction, the extract transferred to a separatory funnel, and the residue rinsed twice with water (2 × 40 mL) and chloroform (50 mL). The aqueous fraction was washed first with hexane (50 mL) and then with ethyl acetate (50 mL). Both hexane and ethyl acetate washings were discarded. Darco (G-60) charcoal (1 g) was added to the aqueous fraction and filtered under suction. The filtrate was concentrated to ~5 mL in vacuo at 60 °C and filtered through a Millipore filter (0.45 μm, Millipore, Waters). After filtration, the pH was adjusted to 0.5 with phosphoric acid and the sample was evaporated to dryness in vacuo at 60 °C. The dried samples were stored under vacuum in a desiccator containing phosphorus pentoxide.

Derivatization. The derivatization reactions utilized in this procedure are based on those described by Deyrup et al. (1985). The flask containing the residues of GLYPH and AMPA from the previous extraction was equipped with a Claisen condenser and an anhydrous calcium chloride guard tube. A gentle stream of dry nitrogen was passed through the system. Trifluoroacetic anhydride (2 mL) followed by trifluoroethanol (1 mL) were added. The mixture was then refluxed for 90 min in an oil bath at 80 °C. The excess reagents were removed by a gentle stream of nitrogen at 40 °C. The derivatives were cooled in an ice–water bath, water (5 mL) was added, and the contents were transferred to a 125-mL separatory funnel with water (5 mL) and then chloroform (60 mL) as rinse. After the mixture was shaken for ~1 min, the chloroform layer was collected and the aqueous layer was extracted two more times with chloroform (2 × 60 mL). The combined chloroform extract was dried over anhydrous sodium sulfate (2-cm bed) followed by the removal of the solvent in a vacuum rotary evaporator at 60 °C. The residue was dissolved in ethyl acetate and injected into the gas chromatograph. The derivatized sample was stable for at least 2 weeks.

Runoff water samples were allowed to thaw at 4 °C. They were then evaporated to 100 mL and extracted according to the method described above.

The deposit sheets were also thawed at 4 °C and the contents eluted with water, concentrated to 100 mL, and extracted as above.

Gas Chromatographic Analysis. The gas chromatographic analysis was conducted on a Shimadzu GC-9A gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a nitrogen–phosphorus detector. The chromatographic column was Ultra-bond 20SE on 80/100-mesh support (Ultra Scientific, Hope, RI): 1.8-m glass; 3-mm i.d. The operating parameters of the gas chromatographs were as follows: detector temperature, 250 °C; column temperature, 150 °C; injector temperature, 250 °C; gas flow rate, nitrogen 50 mL/min (ultra high purity), hydrogen 4 mL/min (pure), and air 175 mL/min (high purity). Samples were sandwiched between two injections of the same standard. A detector fluctuation of ±10% was considered acceptable, and a linear range between 50% and 200% of the average of standards was used in the quantification. Residue concentrations were quantified by the comparison of peak areas to the average peak area of standards run before and after each sample. A Shimadzu C-R3A data processor was used for quantification.

Fortification. Aliquots of prespray residue free soil were placed in a 250-mL screw-cap bottle. Appropriate aliquots of previously prepared solutions containing both GLYPH and AMPA were added to provide fortification levels of 1, 0.48, and 0.096 ppm for GLYPH and 0.5, 0.18, and 0.035 ppm for AMPA, respectively. The bottles were capped, manually shaken to ensure thorough mixing, and stored at −20 °C for 24 h to simulate residue sample storage conditions. The fortification of water was performed in the same manner.

Table V. Residue Values of Glyphosate and Its Metabolite AMPA from the Sand Persistence Site

days post-spray	soil layer[a]	glyphosate residue, μg ± SD	AMPA, μg ± SD
0	O	707.3 ± 61.4	ND
	M1	ND	ND
	M2	ND	ND
1	O	1289.2 ± 208.6	ND
	M1	ND	ND
	M2	ND	ND
6	O	1005.2 ± 102.3	59.9 ± 3.6
	M1	ND	ND
	M2	ND	ND
14	O	741.2 ± 121.9	89.7 ± 49.6
	M1	58.6 ± 48.6	ND
	M2	ND	ND
49	O	214.2 ± 112.3	23.4 ± 7.1
	M1	ND	ND
	M2	ND	ND
96	O	75.4 ± 7.0	52.1 ± 5.5
	M1	ND	ND
	M2	ND	ND
335	O	20.4 ± 6.0	11.8 ± 3.8
	M1	ND	ND
	M2	ND	ND
691	O	ND	ND
	M1	ND	ND
	M2	ND	ND
762	O	ND	ND
	M1	ND	ND
	M2	ND	ND

[a] Key: O = organic; M1 = organic to 15 cm; M2 = 15–30 cm; ND = not detectable; limit of detection 0.05 and 0.01 ppm (μg/g) for glyphosate and AMPA, respectively.

RESULTS AND DISCUSSION

Recovery Efficiency. Recoveries for GLYPH from fortified soil and water samples were as follows: 77.8 ± 8.6% in organic matter; 52.1 ± 1.9% in the clay; 47.7 ± 2.3% in the sand; 94.2 ± 3.6% in water. Similarly recoveries for AMPA were 65.0 ± 4.5% in organic matter, 50.1 ± 1.3% in the clay, 42.6 ± 3.1% in the sand, and 91.1 ± 5.4% in water.

Persistence. In the case of the sand persistence site, the time required for dissipation of GLYPH to less than 50% of the highest residue values observed was 24 days. After 78 days postspray, these values were reduced to below 10% (Table V). Throughout the observation period GLYPH was found to remain in the organic layer. A general trend of dissipation of GLYPH residues with time is evident. The results showed that the recovery of GLYPH at 0 time was lower than expected. No adequate explanation for this could be found. However, two important reasonings could be considered for this high variability: (i) Immediately after spraying the chemical reaching the ground was of unilayer in thickness and sufficient time had not been allowed for its translocation within the soil. In this situation the collection of the soil cores could have resulted in a loss of the herbicide via sorption to plastic bags or instruments used. (ii) Certain vegetation cover still remaining on the ground prevented the total spray from reaching the ground.

Leaching. It was evident from the results of the persistence site that the average GLYPH residue in the upper organic layer was always more than 95% of the total present in the soil core at any time (Table VI). Results

Table VI. Mean Percentage Distribution of Glyphosate in Layers[a] from the Sand Persistence Site

days postspray	organic layer, %	organic 15 cm (M1), %	15–30 cm (M2), %
0	100.00	0.00	0.00
1	100.00	0.00	0.00
6	100.00	0.00	0.00
14	95.16	4.84	0.00
49	100.00	0.00	0.00
96	100.00	0.00	0.00
335	100.00	0.00	0.00
691	–[b]	–	–
762	–	–	–

[a] Calculated as the basis of total amount in the cores at each sample time. [b] Not determined because no detectable amounts of glyphosate were found from the analyzed samples for those two sampling dates.

Table VII. Glyphosate Residue Values (μg) from the Clay Mobility Site[a]

days postspray	distance, m				
	0	3	6	9	12
0	1337.9	ND	ND	ND	ND
2	918.4	ND	ND	ND	ND
7	1185.2	ND	ND	ND	ND
14	273.0	ND	ND	ND	ND
28	1059.5	ND	ND	ND	ND
43	373.0	ND	ND	ND	ND
78	162.4	ND	ND	ND	ND
125	NA	ND	ND	ND	ND
365	17.1	ND	ND	ND	ND
721	ND	ND	ND	ND	ND
792	ND	ND	ND	ND	ND

[a] Only the top strip was sprayed. Key: ND = not detectable; NA = not available; limit of detection = 0.05 ppm (μg/g).

also indicate that GLYPH has a very limited potential to leach vertically through the soil column under the conditions of the study. Overall the study shows that no detectable residue of GLYPH or AMPA could be found either in the organic to 15 cm or 15–30 cm levels; thus this chemical can be considered as essentially nonleachable under the conditions of the experiment.

Mobility. On the basis of the results of this study on the clay mobility site, there was no evidence of lateral movement of the GLYPH down the 8° slope in either runoff water or through subsurface flow as no GLYPH in the quantifiable range (0.1 ppm) could be detected either at a distance 3 m from the top of the application zone or in the runoff water collected in the trench (Table VII).

Metabolite AMPA. The overall trend of metabolite AMPA formation showed that within the observation period as GLYPH concentration decreased, concentration of metabolite AMPA increased and then decreased, indicating that it is a nonpersistent metabolite. The maximum amount of metabolite AMPA formation was 10.24% with respect to GLYPH concentration. In general, the concentrations of metabolite AMPA in these sites were very low in comparison with GLYPH (Table V).

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