Uptake and persistence of the herbicide glyphosate (Vision®) in fruit of wild blueberry and red raspberry

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Field studies on the uptake and persistence of glyphosate (*N*-(phosphonomethyl)glycine) on wild blueberry (*Vaccinium myrtilloides* Michx.) and red raspberry (*Rubus strigosus* Michx.) under boreal forest (Matheson, Ont.) conditions were undertaken. Uptake studies indicated that less than 10% of glyphosate penetrated the fruit in the first 9 h postapplication. Results of the persistence studies showed a gradual decline in residue levels with time. Times to 50% dissipation for glyphosate residue as determined by curvilinear regression analyses were <20 days (95% confidence limit of 8–26 days) and <13 days (95% confidence limit of 6–14 days) for blueberry and raspberry fruit, respectively. Initial residue levels dissipated to approximately 4 and 6% after 61 and 33 days for the blueberry and raspberry, respectively. Results also showed that at no time during the study period did glyphosate levels in either substrate dissipate to below the maximum permissible residue level (0.01 ppm) as established by Health and Welfare Canada.

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Des essais au champ ont porté sur l'assimilation et la persistance du glyphosage (*N*-(phosphonométhyle)glycine) sur la Myrtille sauvage (*Vaccinium myrtilloides* Michx.) et la Framboise rouge (*Rubus strigosus* Michx.) en forêt boréale (Matheson, Ontario). Les études d'assimilation ont montré que moins de 10% du glyphosate pénétrait les fruits durant la période de 9 h suivant l'application. Les résultats des études sur la persistance ont montré un déclin graduel des niveaux de résidus avec le temps. La période provoquant une dissipation à 50% des résidus de glyphosate, telle que déterminée au moyen d'analyses de régression curvilinéraire, a été <20 jours (95% de probabilité de 8 à 26 jours) et <13 jours (95% de probabilité de 6 à 14 jours) pour la myrtille et la framboise, respectivement. Les niveaux initiaux de résidus se sont dissipés à environ 4 et 6% au bout de 61 et de 33 jours pour la myrtille et le framboisier, respectivement. Les résultats ont aussi montré qu'en aucun moment durant la période sous étude le niveau de glyphosate ne s'est dissipé sous le niveau de résidu maximal permis (0,01 ppm) dans l'un ou l'autre substrat tel qu'établi par Santé et Bien-être social Canada.

[Traduit par la revue]

Introduction

Glyphosate (Vision®) (formerly Roundup®) is a broad spectrum herbicide and widely used in silviculture for the control of competing vegetation. Two common competing species in the Ontario boreal forest are wild blueberry (*Vaccinium myrtilloides* Michx.) and red raspberry (*Rubus strigosus* Michx.). Chemical applications of glyphosate are typically made in mid- to late-summer when wild fruit are fully ripe. Although treated areas are posted, contaminated fruit may be consumed by various wildlife species and picked by humans for personal consumption or commercial sale. Data pertaining to the levels of glyphosate residue to be expected in berries following herbicide applications and the persistence of such residues in ripe, edible berries under conditions typical of the boreal forest ecosystem of Canada are lacking. Without a thorough understanding of these aspects, assessment of the environmental hazard associated with contaminated fruit is questionable. The purpose of this work was to establish an analytical method to determine glyphosate and its metabolite, aminomethylphosphonic acid (AMPA), residues from treated fruit of wild blueberry and red raspberry and to determine the uptake and persistence of glyphosate into the fruit of those berries under field conditions.

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Materials and methods

Site selection and preparation

Two sites were selected in the district of Cochrane, Ontario, for these studies. The blueberry site was located 40 km east of Matheson, Ontario, and 15 km south of Highway 101 in Harker Township (48°30′N, 79°W) near Ghost Mountain. The raspberry site was located in Lamplugh Township (48°35′N, 79°W), 40 km east of Matheson and 17 km north of Highway 101 and Ghost Mountain. Because these sites differ in their physical nature, their site preparation will be discussed separately.

Blueberry site

Six plots (2 × 20 m), separated by buffer zones with a minimum width of 5 m, were selected and designated as Hark A–F. Criteria for this selection were the abundance of berry crop and a minimum amount of other vegetation, namely small jack pine trees. The site was prepared by

TABLE 1. Assessment of deposit following glyphosate application to the blueberry site

	Volume applied (L/ha)	Rate applied (kg/ha)	Rate applied from Petri dish[a] Mean ± SD (kg/ha)	%CV
Hark A	433.75	2.316	2.290 ± 0.144	6.3
Hark B	388.75	2.075	1.660 ± 0.069	4.2
Hark C	453.75	2.423	1.919 ± 0.136	7.1
Hark D	458.75	2.449	1.905 ± 0.119	6.2
Hark E	391.25	2.089	2.657 ± 0.151	5.7
Hark F	316.25	1.688	1.420 ± 0.072	5.1
Average	407.08	2.173 ± 0.287 (13.2)[b]	1.975 ± 0.443	22.4

[a]An average of the three replicate Petri dishes per application zone.
[b]% of carrier volume is given in parentheses.

TABLE 2. Assessment of deposit following glyphosate application to the raspberry site

	Volume applied (L/ha)	Rate applied (kg/ha)	Rate applied from Petri dish[a] Mean ± SD (kg/ha)	%CV
Lamp A	441.25	2.356	2.100 ± 0.120	5.7
Lamp B	266.25	1.420	1.065 ± 0.052	4.9
Lamp C	348.75	1.860	1.780 ± 0.064	3.6
Lamp D	466.25	2.480	2.720 ± 0.133	4.9
Lamp E	373.75	1.990	1.587 ± 0.043	2.7
Lamp F	378.75	2.020	1.446 ± 0.077	5.3
Average	379.17	2.021 ± 0.377 (18.6)[b]	1.783 ± 0.574	32.2

[a]An average of the three replicate Petri dishes per application zone.
[b]% of carrier volume is given in parentheses.

removing logs and cutting small trees to ensure unobstructed chemical application. Small trees were cut back to within 1 m of the ground wherever necessary. Glass Petri dishes (153.8 cm^2) were placed within the plots on wooden posts at approximately the height of the blueberry plants to determine the actual amount of chemical deposited on the berries. Plants from an adjacent but unsprayed area were selected as a check control.

Raspberry site

Six plots (2 × 20 m), separated by buffer zones with a minimum width of 5 m, were selected and designated as Lamp A–F. Criteria for the selection were the presence of canes at least 2 years old and the abundance of berry crop. Preparation of the sampling plot consisted only of the cutting back of any tall weed or sapling cover to the height of the raspberry growth. Glass Petri dishes (153.8 cm^2) were again placed within the plots on wooden posts at approximately the height of the raspberry plants to determine the actual amount of chemical deposited on the berries. Plants from an adjacent but unsprayed area were selected as a check control. All plots were marked with red and white colored posts, indicating that the site was an experimental area, with berry picking prohibited.

Chemical application

Chemical applications were made on August 8, 1985, at 05:00 to replicate strips in both the blueberry and raspberry sites. Glyphosate, *N*-(phosphonomethyl)glycine, was applied as an aqueous solution of Vision® (35.6% active ingredient (a.i.)) with a Pestex® backpack sprayer (boom length, 2 m; number of nozzles, four; nozzle type, Tee Jet AL 8004; capacity of reservoir, 2000 mL) using compressed air (200 kPa) as a propellant. The height of raspberry plants at the time of application was 1.2 m above the ground, and the boom was held ~30 cm above the raspberry canopy. Carrier volume rate was 500 L/ha. Because of the limitations of the delivery rates and speed of the hand-operated sprayer, a relatively large volume rate (500 L/ha instead of 35 L/ha) of spray was perforce employed.

The target chemical application rate was 2 kg a.i. per hectare. Actual application rates were determined using glass Petri dishes (153.8 cm^2) as artificial deposit collectors. Replicate Petri dishes were placed inside the experimental strips at the height of the plants. Immediately after application, the Petri dishes were collected, labelled, and frozen until analysis. Sprayer reservoir volumes were monitored before and after spraying of each strip, which allowed calculation of rate of application. All volume measurements were made using a 2-L graduated cylinder. Application rates for both the sites are given in Tables 1 and 2.

Sampling

Samples of ripe berries were collected at 0 and 9 h and at 1, 2, 13, 20, 33, and 61 days from the date of spraying. Samples selected at random along the length of the plot were collected using disposable surgical gloves to prevent contamination. Fresh weights of samples were determined using a triple beam balance immediately following collection. The

TABLE 3. Recovery efficiency for glyphosate and AMPA from blueberries and red raspberries

	Fortification ppm (μg/g)		% recovery ± SD (%CV)	
	Glyphosate	AMPA	Glyphosate	AMPA
Blueberries	1.06	0.41	57.52 ± 2.09 (3.64)	108.56 ± 6.29 (5.79)
	0.11	0.04	102.46 ± 4.69 (4.58)	91.03 ± 3.18 (3.49)
	0.05	0.02	51.58 ± 3.86 (7.48)	100.58 ± 5.19 (5.16)
Raspberries	1.06	0.41	62.91 ± 2.95 (4.69)	98.79 ± 10.69 (10.83)
	0.11	0.04	72.89 ± 4.43 (6.07)	103.89 ± 3.28 (3.16)
	0.05	0.02	97.87 ± 7.30 (7.46)	85.06 ± 3.52 (4.14)

NOTE: There were 3 and 4 samples per concentration for blueberries and raspberries, respectively. Limits of detection for glyphosate and AMPA were 0.025 and 0.01 ppm, respectively.

samples collected between 0–9 h were placed in preweighed collanders (diameter, 11.5 cm). The collanders were supported over plastic funnels and their contents washed copiously with distilled water that was collected in plastic bottles. The washings together with washed fruit were stored frozen until analysis. These samples were used for the uptake study. At the same time, unwashed berries were collected for use in the persistence study. Fruit from an adjacent but unsprayed area were sampled as a control. Samples were brought to the laboratory in Styrofoam containers packed with ice. On arrival at the laboratory, samples were frozen at −20°C until analysis.

Weather

Weather conditions were monitored during the 7 days immediately postspray. There was no rainfall during the first 3 days, whereas 6 mm fell on the 4th day and 7 mm on the 6th day. During this period, the temperature averaged 21°C ± 6°C. Unfortunately the rain periods fell during a nonsampling period and therefore no attempt to correlate weather to residue decay could be made. This is a factor, however, in negating any initial loss of herbicide by rain in the uptake study, as no rain fell during the time of that study.

Analytical methodology

A method has been standardized for the quantitative extraction and analysis of glyphosate and its metabolite AMPA from berries. This method involves water extraction, removal of pigments by charcoal treatment followed by column chromatography using cation exchange resin for removal of sugar, and a single-step derivatization reaction.

Extraction and cleanup

A representative berry sample (10 g) was placed in a Waring blender and homogenized with a mixture of water and chloroform (100:50 v/v). The resulting slurry was filtered on a Buchner funnel and the filtrate quantitatively transferred to a 500-mL separatory funnel using several small volumes of water as rinse. After stabilization, the phases were allowed to separate and the chloroform layer was discarded. The aqueous fraction was partitioned with *n*-hexane (50 mL) and ethyl acetate (50 mL), and the organic fractions were discarded. The aqueous fraction was concentrated (~40 mL) and treated with charcoal (0.8 g, Darco G-60) for the removal of the anthocyanin pigments, and the resultant slurry was filtered through a Buchner funnel. The filtrate was concentrated (16 mL) in a vacuum rotary evaporator at 60°C and the pH adjusted to 2.1 with 6 M HCl. This was further concentrated to 4 mL and applied to a Dowex 50W-X8 (H^+ form) cation exchange column (2.2 × 6.5 cm) equilibrated in pH-2.1 water (Guinivan et al. 1982). The first fraction (21 mL), which contained the sugars, was discarded and the eluent changed to deionized water (pH 7.0). The next fraction (225 mL), which contained the herbicide and metabolite, was collected and concentrated in a vacuum rotary evaporator at 60°C followed by evaporation to dryness under nitrogen. The dried samples were stored overnight under vaccum (5 mm Hg) in a desiccator that contained phosphorous pentoxide.

The contents of the Petri dishes for artificial deposit collector analyses were thawed, eluted with water (200 mL), concentrated to 100 mL, and extracted as described earlier. Berry washings taken in the field were concentrated to 100 mL and extracted as described earlier.

Derivatization

The derivatization reaction utilized in this procedure is based on that described by Deyrup et al. (1985). The flask containing the residue of glyphosate and AMPA from the previous extraction was equipped with a Claisen condensor and anhydrous calcium chloride guard tube, and a gentle stream of dry nitrogen was passed through the system. Trifluoroacetic anhydride (TFAA) (4 mL) followed by trifluoroethanol (TFE) (2 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 50°C, and the residue was dissolved in ethyl acetate and analyzed by gas chromatography. Confirmation of the identity of the volatile derivatives produced by these reactions was established by electron impact as well as chemical ionization mass spectroscopy.

Gas chromatographic analysis

The gas chromatographic analysis was conducted on a Shimadzu GC-9A gas chromatograph equipped with a N/P detector and with a 1.8-m column of Ultra-bond 20 SE 80/100, with nitrogen (50 mL/min) as the carrier gas. Column temperature was 150°C.

Field samples were injected alternately with external standards. Using a Shimadzu C-R3A data processor, residue concentrations were quantified by the comparison of peak areas to mean peak areas of standards run before and after each analytical sample. A detector fluctuation of ± 10% for standard response was considered acceptable. The linear

TABLE 4. Uptake of glyphosate in blueberry and raspberry fruit immediately after spray application

	Hours postspray	Wt. (g)	Total μg in the washings		Total μg in washed berries		ppm in the washings (μg/g)		ppm in the washed berries (μg/g)	
			Glyphosphate	AMPA	Glyphosphate	AMPA	Glyphosphate	AMPA	Glyphosphate	AMPA
Blueberry	0	39.8	323.9	ND	ND	ND	8.14	ND	ND	ND
	0	45.0	373.5	ND	ND	ND	8.30	ND	ND	ND
	0	33.4	265.5	ND	ND	ND	7.95	ND	ND	ND
	9	42.3	269.5	ND	54.6	ND	6.37	ND	1.29	ND
	9	51.7	381.6	ND	47.4	ND	7.38	ND	0.92	ND
	9	39.9	278.7	ND	41.5	ND	6.98	ND	1.04	ND
Raspberry	0	37.4	768.9	ND	ND	ND	20.56	ND	ND	ND
	0	32.9	649.5	ND	ND	ND	19.74	ND	ND	ND
	0	43.7	881.4	ND	ND	ND	20.17	ND	ND	ND
	9	40.2	735.3	ND	78.4	ND	18.29	ND	1.95	ND
	9	43.4	760.8	ND	72.5	ND	17.53	ND	1.67	ND
	9	-38.7	664.9	ND	72.8	ND	17.18	ND	1.88	ND

NOTE: ND, not detectable; limits of detection for glyphosphate and AMPA were 0.025 and 0.01 ppm, respectively.

range of the detector was 0.05 to 10 ng for glyphosate and 0.01 to 30 ng for AMPA. Limits of detection for glyphosate and AMPA from this method were 0.025 and 0.01 ppm, respectively, for both blueberry and raspberry.

Analytical method validation

Validation studies were undertaken to determine the recovery efficiency and precision of the analytical technique and to detect a correlation between recovery efficiency and residue concentration. For validation studies, blank berry samples (10 g) collected from the field sites were placed in screw-cap bottles, macerated with a stirring rod, and fortified using a mixed standard of glyphosate and AMPA (70.4 and 40.5 ppm, respectively) at the levels listed in Table 3. Validation test samples were manually shaken and equilibrated for a period of 48 h to ensure adequate adsorption of the chemical to the substrate.

Quality assurance program

A quality assurance program involving two fortified check samples processed concurrently with batches of field samples was employed to check day-to-day variability and recovery efficiency of the method. Quality assurance samples were fortified at various concentrations that approximated expected residue levels in the field samples and were processed and analyzed as for field sample analyses.

Statistical analysis

An analysis of variance was conducted from the data obtained from blueberry and raspberry sites to determine if results were statistically significant. Polynomial regression analysis was also performed on data, and the line of best fit was determined. The mathematical model for these regression analyses are represented by

$$\text{Residue} = a + (b_1 \times \text{days}) + (b_2 \times \text{days}^2)$$

where

Residue = residue value from blueberry and raspberry samples after treatment in mg/kg.

a = Y-intercept

b_1 and b_2 = regression coefficients for days and days^2, respectively

The above regressions were fitted to the data by the least squares method, SAS (SAS Institute Inc. 1985). The 95% confidence limits for the regression lines were also plotted, and the regression equations were used to calculate times for 50% dissipation (DT_{50}).

Results and discussion

Analytical method validation results

The results of the recovery data (Table 3) indicate that the recovery of glyphosate and AMPA from blueberry and raspberry was concentration dependent. This concentration dependence was reproducible over several subsequent trials, but no adequate explanation for this phenomenon could be found. These results negated the use of a universal preanalysis correction factor. Therefore, residue concentrations reported were corrected for analytical efficiency based on mean recovery determined from two quality assurance samples that were analyzed concurrently with batches of five field samples. The recovery efficiencies of glyphosate and AMPA from water were 90.23 and 85.25%, respectively (Roy et al. 1987).

Deposit distribution

Deposit data are presented in Tables 1 and 2. Overall results showed that the estimates of initial glyphosate deposits calculated mathematically from measured sprayer volumes agree closely with empirical residue data as determined by residue analyses conducted on artificial deposit collectors (Petri dishes).

Uptake study

The data from this study (Table 4) indicate that the berries of each species absorb the herbicide at different rates. For blueberry, the figure was 13.63 ± 2.94%, whereas for raspberry only 9.39 ± 0.62% had been taken up 9 h after spray of glyphosate. These uptake data were calculated from the analyses of berry washings and washed berries at 0 and 9 h postspray.

Persistence

Results of the persistence study are presented graphically in Figs. 1 and 2 for blueberry and raspberry, respectively.

TABLE 5. Residue levels of glyphosate and AMPA in blueberry and red raspberry collected from boreal forests of Ontario

	Days postspray[a]	Glyphosate (mg/kg)		AMPA (mg/kg)	
		Mean ± SD	%CV	Mean ± SD	%CV
Blueberry	0	7.94 ± 0.678	8.54	ND	—
	1	6.60 ± 0.708	10.73	ND	—
	2	5.66 ± 1.210	21.38	0.055 ± 0.017	30.91
	13	3.73 ± 0.535	14.34	0.051 ± 0.009	17.65
	20	2.50 ± 0.524	20.96	0.031 ± 0.010	32.26
	33	1.23 ± 0.248	20.16	ND	—
	61	0.19 ± 0.035	18.13	ND	—
Raspberry	0	19.49 ± 2.110	10.83	ND	—
	1	18.25 ± 2.570	14.08	ND	—
	2	17.12 ± 0.990	5.78	0.102 ± 0.024	23.53
	13	5.55 ± 0.880	15.86	0.089 ± 0.031	34.83
	20	3.39 ± 0.420	12.39	0.033 ± 0.008	24.24
	33	1.22 ± 0.122	10.00	0.024 ± 0.004	16.67
	61	NA	—	NA	—

NOTE: Data corrected for glyphosate and AMPA recovery efficiencies. ND, not detectable; limits of detection for glyphosate and AMPA were 0.025 and 0.01 ppm, respectively. NA, not available; no berries on the plantation.
[a]Zero days postspray was August 8, 1985.

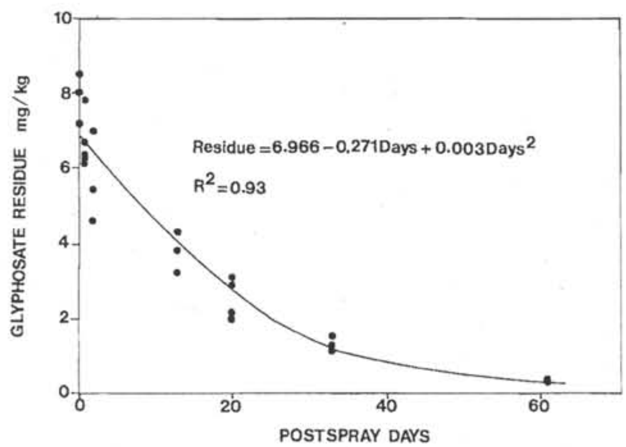

FIG. 1. Dissipation in glyphosate residue from blueberry site.

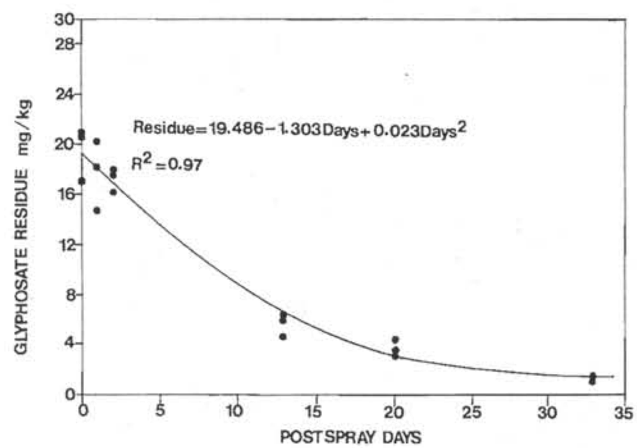

FIG. 2. Dissipation in glyphosate residue from raspberry site.

Actual numerical values for mean glyphosate and AMPA concentrations in the two fruit types at specific sampling times are presented in Table 5. Initial mean residue values in blueberry (7.94 ppm) were lower than those for raspberry (19.49 ppm). It is important to note that application equipment and spray parameters as used in this study are different from those of typical operational chemical applications for glyphosate, and thus the initial residue data should not be considered as residue levels that would be expected from operational spraying of glyphosate. However, the data do clearly indicate declining residue levels of glyphosate with time (Figs. 1 and 2). Transient increases in AMPA levels suggest that microbial and (or) metabolic degradation actually takes place in or on the fruit (Table 5). Curvilinear regression analyses for glyphosate residues with time were characterized by excellent R^2 values (0.92 and 0.96, respectively) and highly significant F values for slopes ($P > F = 0.0001$). From the regression analyses, DT_{50} values were estimated as <20 days (95% confidence limit of 8–26 days) and <13 days (95% confidence limit of 6–14 days) for blueberry and raspberry fruit, respectively. Under the conditions of this study, residues remained above detectable levels and above the maximum permissible residue level (0.01 ppm) as established by the Health and Welfare Canada, Food and Drug Regulation (1980).

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