

The presence of glyphosate in forest plants with different life strategies one year after application

Lisa J. Wood

Abstract: Persistent nonlethal doses of glyphosate in plant tissue may have implications for the edible and (or) medicinal use of native plants. This study investigated native plants growing in northern British Columbia, Canada, to determine glyphosate presence and location within tissue in select species of traditional-use value with different life strategies. Perennial herbaceous and woody plants were collected one year after forestry-based applications of glyphosate in the Peace River Region of British Columbia. Shoot, fruit, and root portions of select species were analyzed for glyphosate and aminomethylphosphonic acid (AMPA) residues using HPLC–IPCMS. Glyphosate residues were found one-year after application. The highest and most consistent levels of glyphosate and AMPA were found in herbaceous perennial root tissues, but shoot tissues and fruit were also shown to contain glyphosate in select species. Levels found in some cases were greater than expected. Findings indicate the ability of glyphosate to be stored in root structures of perennial plants during dormancy periods and move up to shoot and fruit portions in years following applications in some species. Further investigation is required to determine the timeline associated with glyphosate presence in plant tissues.

Key words: traditional-use plants, herbicide, persistence, translocation, functional traits.

Résumé : Des doses persistantes non létales de glyphosate dans les tissus végétaux peuvent avoir des implications pour l'utilisation de plantes indigènes comestibles ou médicinales. Cette étude consistait à examiner des plantes indigènes qui croissent dans le nord de la Colombie-Britannique, au Canada, pour déterminer la présence du glyphosate et l'endroit où il s'accumule dans les tissus d'espèces de choix ayant une valeur liée à leur usage traditionnel ainsi que différentes stratégies de vie. Des plantes herbacées et ligneuses pérennes ont été collectées un an après des applications de glyphosate en forêt dans la région de la rivière Peace en Colombie-Britannique. Des portions de pousse, de fruit et de racine d'espèces de choix ont été analysées pour la présence de résidus de glyphosate et d'acide aminométhylphosphonique (AAMP) par CLHP–IPCMS. Des résidus de glyphosate ont été détectés un an après l'application. Les niveaux les plus élevés et les plus constants de glyphosate et d'AAMP ont été observés dans les racines de plantes herbacées pérennes, mais les pousses et les fruits contenaient aussi du glyphosate chez les espèces de choix. Les niveaux retrouvés dans certains cas étaient plus élevés que ceux qui avaient été anticipés. Les résultats indiquent que le glyphosate peut être emmagasiné dans les structures racinaires des plantes pérennes durant les périodes de dormance et se déplacer vers les pousses et les fruits dans les années suivant les applications chez certaines espèces. D'autres études sont nécessaires pour déterminer la chronologie associée à la présence de glyphosate dans les tissus végétaux. [Traduit par la Rédaction]

Mots-clés : plantes à usage traditionnel, herbicide, persistance, translocation, traits fonctionnels.

Introduction

Glyphosate is an active ingredient of broad-spectrum herbicides commonly used to treat unwanted vegetation in forestry and industrial settings (Henderson et al. 2010). Herbicides have been used annually in forestry (and other industrial) operations in British Columbia (BC) for over 30 years. The total forested area of BC over which herbicides have been applied is approximately 650 000 ha, with an average since year 2000 of approximately 17 000 ha·year⁻¹ (British Columbia Ministry of Forests, Lands, and Natural Resource Operations 2016), the majority of which are glyphosate-based herbicides. This does not include agricultural applications. Treatments in the northern interior of BC make up 90% of all aerially applied herbicide applications and 57% of all ground-based spraying in BC annually (Govindarajulu 2008). In forestry settings, aerial application of glyphosate may result in spray drift and incomplete application to plants in the under-

story. This partial treatment results in plants surviving the application, which may then lead to altered phenotypic expression in response to chemical presence and corresponding plant localization and (or) isolation and storage of glyphosate, genetic mutation, or metabolic action (Sammons and Gaines 2014). In plants surviving glyphosate treatment, the timeline of low-level glyphosate persistence and plant-specific responses over time are unknown.

After application, glyphosate degradation in the environment is dependent on the substrate upon which it interacts, whether it be plant tissue, animal tissue, soil, water, or air (Bergström et al. 2011; Coupe et al. 2011). When glyphosate comes into contact with soil, it binds to organic matter, iron, and aluminum and especially clay particles (Miles and Moye 1988). Adsorption of glyphosate to soil particles happens within the first hour after application (Canadian Council of Ministers of the Environment (CCME) 2012).

Received 8 August 2018. Accepted 18 December 2018.

L.J. Wood. * Spectrum Resources Group Inc., 3810 18th Ave., Prince George, BC V2N 4V5, Canada.

Email for correspondence: lisa.wood@unbc.ca.

*Present address: University of Northern British Columbia, 3333 University Way, Prince George, BC V2N 4Z9, Canada.

Copyright remains with the author(s) or their institution(s). Permission for reuse (free in most cases) can be obtained from [RightsLink](https://www.copyright.com/).

pH affects the solubility of glyphosate ions in solution; soils with a higher pH may have more freely moving glyphosate ions than more acidic soils (Miles and Moye 1988; CCME 2012). When bound to clay or metals in the soil matrix, glyphosate is somewhat stabilized (Al-Rajab and Hakami 2014). Over time, glyphosate molecules within soil or water are broken down by microorganisms (CCME 2012). The speed at which glyphosate molecules degrade is dependent on the presence of microbes; the more plentiful the microbial population capable of glyphosate degradation, the faster the breakdown occurs. Residual glyphosate that persists for any given time may indicate slower activity by the microbes responsible for its degradation (Laitinen et al. 2006; Stenrød et al. 2005), and in these cases, the risks associated with persistent glyphosate molecules are unclear (Relyea 2005; Benachour et al. 2007; Kissane and Shephard 2017).

Glyphosate breakdown in living plants is variable and much is not understood. It is clear that plants surviving glyphosate treatment contain levels of glyphosate after application (Ando et al. 2002); however, thorough research remains to be conducted across environmental gradients on the length of persistence of glyphosate after application, especially at low levels beyond the half-life period, where in the plant body glyphosate may be isolated and stored, and how these factors are related to plant functional traits.

Glyphosate, or *N*-(phosphonomethyl)glycine ($C_3H_8NO_5P$), translocates rapidly into plants due to its solubility in water (CCME 2012). Once present in a plant system, glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase in the shikimic acid pathway, which reduces amino acids that are used in plant growth and other biochemical processes (Duke and Powles 2008). Plants that are not killed after this process are usually reduced in health and growth function (Reddy et al. 2008). Glyphosate has a very low potential to bioaccumulate in animal tissue due to its low octanol to water partition coefficient (CCME 2012), indicating that it partitions into water and out of fatty acids and lipids (Halsall 2007).

Over time, glyphosate molecules are oxidized to glyoxylate and aminomethylphosphonic acid (AMPA) (Duke et al. 2012). AMPA (CH_6NO_3P) may take up to two years or more to breakdown (Coupe et al. 2011; Wiersema et al. 1997) and together with glyoxylate ($CHOCO_2H$) breaks down into carbon dioxide, formylphosphonate, and methylamine, which then become ammonium, formaldehyde, phosphate, and carbohydrates (CCME 2012). AMPA has similar characteristics to glyphosate from a toxicology perspective (Woodburn 2000), although AMPA has a different mode of action (Reddy et al. 2008). The oxidation of glyphosate to AMPA requires an oxidoreductase gene (GOX), which has never been found naturally in plants, although it is present in bacteria (Howe et al. 2002). GOX genes from bacteria are used in genetically modified corn and soy to produce crops resistant to glyphosate for ease of weed control (Cerdeira and Duke 2006; Hadi et al. 2012).

At times, nontarget plants are subject to a treatment of glyphosate as a result of overspray, spray drift, or simply because of their proximity to a targeted plant in a vegetation management scenario (Schrübbbers et al. 2016). When nontarget plants are sprayed, it is often with low, nontoxic doses, because they did not receive a complete application. Research suggests that plants treated with nontoxic doses of glyphosate may store the glyphosate molecules indefinitely, may translocate glyphosate out of their tissue into the surrounding environment, and (or) may slowly break down glyphosate (Kremer et al. 2005; Henderson et al. 2010; CCME 2012). The value of forest plants as forage or as edible and medicinal plants for people comes into question if plants contain glyphosate.

Unique plant features such as rooting depth, concentration of fine roots, stem height, concentration of parenchyma storage cells, and mechanism of sugar allocation and storage may all contribute to plant exposure to glyphosate applications and ability to

uptake, translocate, store, and (or) metabolize glyphosate ions (Cornish 1992; Wagner et al. 2003; Duke 2011). For example, compare bunchberry (*Cornus canadensis* L.), which has edible berries and is a short, trailing plant, with clasping twistedstalk (*Streptopus amplexifolius* (L.) DC.), which also has edible berries and leaves but grows between 0.5 and 1 m in height. Bunchberry may be in greater contact with soil or possess lower transportation requirements for ions to reach leaves compared with twistedstalk and therefore possess more glyphosate ions in leaves after a ground-based herbicide treatment. Conversely, the taller clasping twistedstalk plant may be more susceptible to drift from aerial applications of glyphosate.

Identifying glyphosate persistence in northern BC is important for the traditional use of plants for food and medicines and to better understand the composition of plants that are food sources for wildlife. Concern has been expressed regarding glyphosate presence and potential unknown interactions in northern environments (Helander et al. 2012). Understanding more about the presence of glyphosate in plants that are used extensively by multiple First Nations groups (Bannister 2006; Turner 2010; Mackinnon et al. 2014) will allow managers to improve practice and better inform public on practices involving glyphosate.

Objectives and predictions

This study examined the presence of glyphosate and its primary metabolite, AMPA, in specific plants located within forestry cut blocks in the Peace River Region of BC, one year after standard operational treatments (Table 1; Fig. 1), to

1. determine if glyphosate and AMPA residues were present in specific plant parts (shoots, roots, berries) after the application of glyphosate on northern sites, and
2. examine for species, plant structure, and perennation type specific trends respecting the presence and (or) concentration of glyphosate and AMPA, when detected.

It was hypothesized that glyphosate translocation would take place upon initial treatments and that woody and herbaceous shoots that came in contact with glyphosate spray would die within one year of spray and would not regrow. It was assumed that live plants sampled one year after application were missed by the treatment due to placement in the canopy structure of the opening. No AMPA was predicted to be found in plant tissue, as no glyphosate was predicted to be in the plants sampled, and there is no known mechanism for the metabolic degradation of glyphosate in plants.

Methods

This study began out of interest by a First Nations community to determine glyphosate presence and activity in local, native plants. In 2013 and 2014, deciduous shrubs were targeted for sampling as they were of interest to traditional berry-pickers. Glyphosate was unexpectedly detected in some deciduous tissues. Following this finding, an expansion of the study was undertaken in 2015 to investigate glyphosate residues in other perennials to determine if the presence of glyphosate residues was similar in herbaceous and woody plants. Therefore, to keep the time-since-application variable consistent, this study occurs over a three-year period of time and investigates glyphosate residue on different cut blocks.

Ten native plant species were targeted for sampling between 2013 and 2015: four herbaceous perennial species, two evergreen perennial species, and four woody shrubs (Table 1). Species were selected for their potential importance as traditional-use plants (Bannister 2006; Turner 2010; Mackinnon et al. 2014).

Study areas

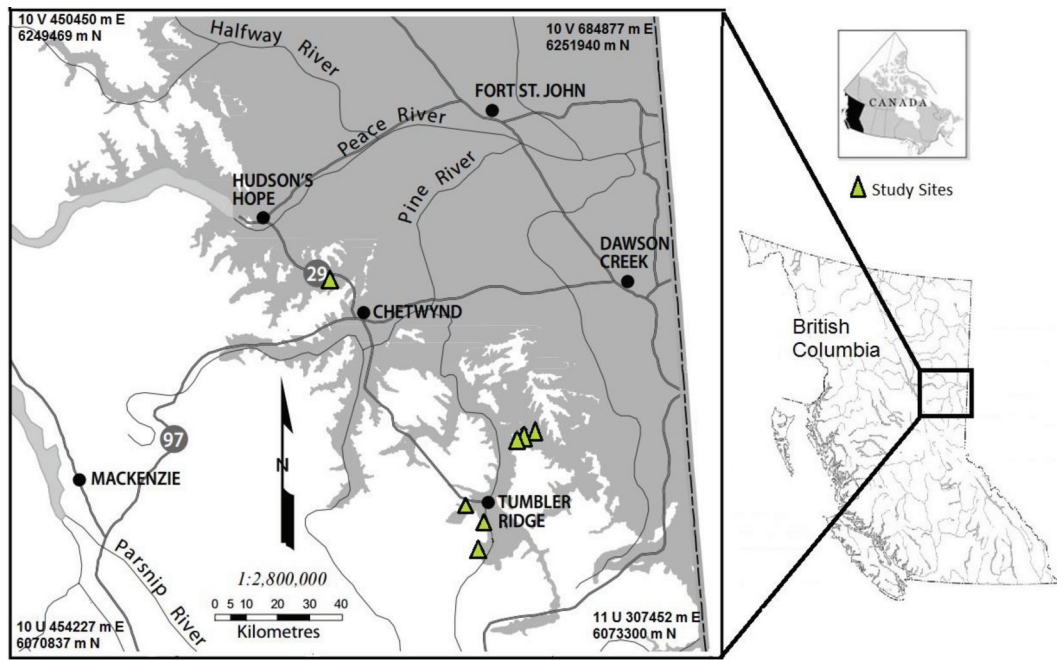
Study areas were selected within the Peace River Region of BC according to local herbicide application schedules. All sites targeted for sampling fell within the Boreal Black and White Spruce

Table 1. Plant samples collected for glyphosate residue analysis in the Peace River Region of British Columbia along with location and date of collection.

Year sampled	Cut-block identification	Soil series ^a	Clay (%); pH range ^a	Glyphosate application year	Species sampled	Plant perennation type	Plant structure sampled
2014	124-4	80% Nose soil series sandy clay loam to sandy loams	5–20; 5.6–7.6	Control	<i>Viburnum edule</i> <i>Rosa acicularis</i> <i>Vaccinium ovalifolium</i> <i>Rubus idaeus</i>	Shrub Shrub Dwarf shrub Shrub	Roots, leaves Roots, leaves Berries Berries
2013	84-1	Moberly Bisequa Gray Wooded loam and clay loams	10–30; 5.1–7.7	2012	<i>Viburnum edule</i> <i>Rosa acicularis</i> <i>Rubus idaeus</i>	Shrub Shrub Shrub	Roots, leaves Roots, leaves Roots, leaves
2014	188-1	Sundance Bisequa Gray Wooded loamy sand and sandy loam	10–20; 5.2–7.6	2013	<i>Rosa acicularis</i> <i>Rubus idaeus</i>	Shrub Shrub	Roots, leaves Berries
2014	130-1	80% Nose soil series sandy clay loam to sandy loams	5–20; 5.6–7.6	2013	<i>Viburnum edule</i> <i>Rosa acicularis</i> <i>Vaccinium ovalifolium</i> <i>Rubus idaeus</i>	Shrub Shrub Dwarf shrub Shrub	Roots, leaves Roots, leaves Berries Berries
2015	635-5, 635-4, 635-4A	70% Pinto soil series and 30% Moberly soil series loamy sand and sandy loam	0; 4.7–7.0	2014	<i>Heracleum lanatum</i> <i>Streptopus amplexifolius</i> <i>Cornus canadensis</i> <i>Pyrola asarifolia</i> <i>Petasites palmatus</i> <i>Galium triflorum</i>	Herbaceous perennial Herbaceous perennial Evergreen perennial Evergreen perennial Herbaceous perennial Herbaceous perennial	Roots, shoots Roots, shoots Roots, shoots Roots, shoots Roots, shoots Roots, shoots

^aReferences for soil information: Farstad et al. (1965), Lord et al. (1977), Alberta Research Council (1980), and Government of British Columbia (2017).

Fig. 1. Overview of locations in the Peace River Region of British Columbia used for plant sampling to detect potential glyphosate and AMPA residues after application. [Colour online.]



moist warm (BWBSmw) biogeoclimatic zone. Sites were further described as mesic to rich in nutrients and mesic to subhydric in moisture regime and ranged in elevation between 830 and 1100 m (DeLong et al. 2011). The soils in the areas sampled were classified as Brunisolic Gray Luvisols and lay above coarse, fragmented morainal till. Soil information was obtained from maps and inventory surveys conducted in previous studies of the areas sampled (Farstad et al. 1965; Lord et al. 1977; Alberta Research Council 1980; Government of British Columbia 2017). In all areas sampled, soil texture and pH levels vary as soil depths and horizons fluctuate (Table 1).

Study sites were treated with the glyphosate-based herbicide product VisionMax™, made by Monsanto Canada Inc., one year

prior to plant sampling to target and remove trembling aspen (*Populus tremuloides* Michx.) competition surrounding plantation conifers (Table 1; Fig. 1). The active ingredient in VisionMax™ liquid formulation is glyphosate, at a product concentration of 540 g acid equivalent·L⁻¹, present as potassium salt. The formula is composed of 49% potassium salt of glyphosate, 10% surfactant mixture, and 41% water. In VisionMax™, the surfactant mixture is proprietary (Monsanto Canada Inc. 2011). Aerial applications were conducted over the treated sites, with the exception of cut block 84-1, which was sprayed with a backpack applicator over only a portion of the block. A prescribed 4 L·ha⁻¹ of VisionMax™ were sprayed once, uniformly over the treatment areas, at a concentration of 8% (4 L chemical formula added to 50 L water), following

standard forestry operational procedures for herbicide application from a helicopter. This standard method relies upon the helicopter pilot to spray the herbicide over the treatment area while maintaining a constant speed and distance from the ground. Aerial herbicide applications are only permitted when wind levels are lower than $8 \text{ km}\cdot\text{h}^{-1}$ to prevent spray drift. Cut block 124-4 was targeted for sampling as a control block (Table 1).

Blocks were sprayed during the second week of August in the years of application (Table 1), with average temperatures at the exact times of application ranging from 12.1 to 19.8 °C. Between 2012 and 2015, average August monthly temperatures for the region were $29.3 \text{ °C} \pm 3.3 \text{ °C}$. The coolest average August temperature for this period was in 2015, with a monthly average of 25.4 °C (Environment Canada 2017). Following herbicide applications, the average winter conditions in the region consisted of 200 to 300 cm of snowpack and temperatures ranging from 2 to -36 °C between November and January (Environment Canada 2017), when plants were dormant.

Plant sampling

Six forest cut blocks were targeted for plant collection one year after glyphosate application. It was assumed that the baseline glyphosate level for untreated areas was zero; a control was used to confirm this assumption for plants collected during the 2014 season. Individual plants from each species were randomly collected over the forest opening, and effort was made to choose plants from across the openings for spatial representation. Three to five individual plants of a targeted species were collected within each cut block, depending on species prevalence. Shoots and roots were collected from highbush cranberry (*Viburnum edule* (Michx.) Raf.), prickly rose (*Rosa acicularis* Lindl.), common cowparsnip (*Heracleum lanatum* Michx.), clasping twistedstalk (*Streptopus amplexifolius* (L.) DC.), bunchberry (*Cornus canadensis* L.), pink wintergreen (*Pyrola asarifolia* Michx.), palmate coltsfoot (*Petasites palmatus* (Aiton) A. Gray), and sweet-scented bedstraw (*Galium triflorum* Michx.). Shoots, roots, and berries were collected from red raspberry (*Rubus idaeus* L.), and only berries were collected from oval-leaf blueberry (*Vaccinium ovalifolium* Sm.). Plant species were obtained from blocks in which they were present.

Plant structures (shoots, roots, fruit) were collected for separate analyses from across the individual plants to obtain a representation of the whole plant rather than just one section of the plant body. New growth was chosen for sampling to avoid vegetation that may have been exposed to treatment at the time of application. All plants targeted were either deciduous woody shrubs, sampling only live leaves and (or) fruit that would have been grown in the season after application, or herbaceous perennials, sampling the entire shoot, which was newly grown in the year of collection. An exception exists in two cases: pink wintergreen and bunchberry can be evergreen plants in some environments (Mackinnon et al. 1999). In the case of these two species, efforts were made, where possible, to choose newly grown leaves for sampling.

Sampled plants were placed in separate bags, labeled with species, area identification, and treatment identification, and frozen until needed. After plant collection, samples were brought back to the University of Northern British Columbia (UNBC), Prince George, BC, where all plants were washed with water thoroughly to remove soil and then oven-dried for 24–48 h at 80 °C in labeled brown paper bags. Dried plant tissues were ground to a fine powder and placed in labeled collection containers. All equipment was washed thoroughly with water between uses to avoid cross-contamination. Plant samples of similar species and parts within each block treatment were combined to form composite samples for analysis, which removed individual-plant bias and provided an average representation of what could be expected to be found for each species on each site. Three composite samples were analyzed per plant structure type per species, except in the case of rasp-

berry and blueberry fruit, where only two composite raspberry samples and one composite blueberry sample were obtained. The composite samples were divided into duplicates for confirmation of results during laboratory analyses. Plants were sent to the UNBC Analytical Chemistry Laboratory and to the University of Guelph Agriculture and Food Laboratory for analyses. Two labs were used for chemical analyses due to the unavailability of the UNBC Analytical Chemistry Laboratory after the first year of study.

Laboratory analysis

Plant samples were tested in 2013 (Table 1) at the UNBC Analytical Chemistry Lab for the presence of glyphosate parent ions and the presence of AMPA. Analysis was performed using high-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICPMS), which is the preferred detection method for glyphosate in Canada due to its reliability (CCME 2012). Plant material was extracted with 100 mmol borate (pH 9.2) for 2 h, and dichloromethane was used to clean the plant extracts. The detection limit for glyphosate and AMPA, based on the analysis procedure followed at the UNBC Analytical Chemistry Lab (HPLC-ICPMS), was 0.02 ppm. The samples returning the result <0.02 ppm (20 ppb) were assigned a value of zero for the purposes of statistical analysis.

The plant samples collected in 2014 and 2015 (Table 1) were tested at the University of Guelph Agriculture and Food laboratory (AFL) for the presence of glyphosate parent ions and the presence of AMPA ions using HPLC. Samples were extracted in an aqueous solution, and the extract was acidified and passed through a Solid Phase Extraction cartridge for analysis using HPLC-MS/MS for glyphosate and AMPA. The method used by the AFL had a detection limit of 5 ppb for 5 g samples.

Statistical analysis

A 95% statistical significance level ($\alpha = 0.05$) was used throughout the analysis. Data were tested for normality using kurtosis and skewness values and through interpretation of histograms. Average glyphosate and AMPA detected over samples collected were deemed non-normal in distribution, skewed significantly to the right. The glyphosate and AMPA detection data were heavily clustered at zero due to the number of samples in which levels were undetected and the dataset consisted of only positive values; therefore, a Tweedie distribution was deemed the most appropriate curve fit. Analyses performed to determine statistically significant differences in glyphosate and AMPA content between plant samples by species, plant structure, perennation type, plant height class, and (or) location were conducted using nonparametric Mann-Whitney and Kruskal-Wallis ANOVA tests. The value of each variable as a model predictor for glyphosate and AMPA content was determined using general linear models (GLMs) with a Tweedie log-link fit; categorical variables including block, species, plant structure, and perennation type were entered as fixed factors, and interactions between factors were analyzed. Statistics were calculated in SPSS (version 24; IBM SPSS Statistics).

Results

No glyphosate or AMPA was detected in any of the control samples. No significant differences in glyphosate and AMPA levels detected were noted between treated sites ($p = 0.631$ and $p = 0.281$, respectively) or between spray treatment dates ($p = 0.291$ and $p = 0.139$, respectively) according to the Kruskal-Wallis test performed. Furthermore, when application date, collection date, and cut block number were fixed factors in GLMs to predict glyphosate or AMPA levels, the variables were shown to have an insignificant effect on the model outcomes. When modeling glyphosate levels with these variables, the omnibus test was insignificant (likelihood ratio $\chi^2 = 9.834$, $p = 0.08$), indicating that only the intercept had a significant effect on the model. Similarly, when modeling

Table 2. Number of samples returning a positive detection for glyphosate and AMPA out of the number of samples analyzed between treatments, as well as the structure of the plant where detection occurred, listed by species.

Species	Frequency of positives for glyphosate detection	Plant structure containing glyphosate	Frequency of positives for AMPA detection	Plant structure containing AMPA
<i>Rosa acicularis</i>	4/4	Roots, shoots	0/4	N/A
<i>Viburnum edule</i>	1/3	Roots	0/3	N/A
<i>Vaccinium ovalifolium</i>	1/1	Berries	0/1	N/A
<i>Cornus canadensis</i>	6/6	Roots, shoots	4/6	Roots, shoots
<i>Streptopus amplexifolius</i>	4/6	Roots, shoots	3/6	Roots
<i>Heracleum lanatum</i>	3/6	Roots	2/6	Roots
<i>Galium triflorum</i>	5/6	Roots, shoots	3/6	Roots, shoots
<i>Rubus idaeus</i>	2/2	Berries	0/2	N/A
<i>Pyrola asarifolia</i>	6/6	Roots, shoots	1/6	Roots
<i>Petasites palmatus</i>	2/6	Roots	2/6	Roots

Note: AMPA, aminomethylphosphonic acid; N/A, not applicable.

AMPA from treatment date and site number, results were insignificant (likelihood ratio $\chi^2 = 7.315$, $p = 0.198$). Therefore, the treated blocks $\times$ year of collection variables were not deemed significant in determination of glyphosate and AMPA residue levels for this study, and blocks were combined for further statistical analysis. It should be noted that due to the variations in year and cut blocks sampled, the effects of these two variables cannot be separated, and there is no way of discerning the individual effect of these two variables.

The average climate variation over the region sampled throughout the duration of the study is reasonably consistent. Average temperatures for August (month of glyphosate application) over the region varied by only 8 °C between 2012 and 2015, and the duration was also consistently relatively dry. The month of August received fewer than 10 days with greater than 1.0 mm of rainfall in each year (3.5 days in 2012, 7 days in 2013, 2.5 days in 2014, and 8 days in 2015) (Environment Canada 2017). Translocation of glyphosate is likely slower during hotter, drier periods than during cooler, wetter periods due to changes in evapotranspiration mechanisms and general water movement through plants (Sharma and Singh 2001). Because a large amount of climatic variation was not observed over the study period, variation in translocation due to this factor was hypothesized to be low. Furthermore, growing conditions are somewhat consistent over the region sampled despite some site-level variation. Elevations varied by less than 300 m, and sites were all in the BWBSmw biogeoclimatic zone. Soil types were consistently slightly acidic, as would be found in a coniferous-dominated environment.

Glyphosate concentrations detected varied by species only in shoot portions (Kruskal–Wallis test, $p = 0.022$) (Table 2). Distributions of average glyphosate in root structures across categories of species and average AMPA in all structures across species were not statistically different. Glyphosate was detected in shoots of 12 of 23 plant samples across treatment areas; where detected, average levels ranged from 76.5 to 1050 ppb. AMPA was detected in the shoots of only 3 of 23 samples (Table 2); average levels detected ranged from 15.5 to 47.5 ppb (Fig. 2).

Across all species, the roots of the plants analyzed showed significantly higher levels of glyphosate and AMPA than the shoot structures sampled (Mann–Whitney test, $p < 0.001$ for both glyphosate and AMPA by plant structure). Glyphosate was detected in roots of 20 of 22 samples analyzed, with concentrations ranging from 145 to 4350 ppb. AMPA was detected in 13 of 22 samples (Table 2), with concentrations detected in roots ranged from 28 to 210 ppb (Fig. 2).

Of samples in which glyphosate and AMPA were detected, herbaceous species with shorter forms (<50 cm height) were shown to have higher levels of glyphosate than woody shrubs with taller

forms (>50 cm height) (Mann–Whitney U test, $p = 0.053$) (Fig. 2). Herbaceous plants with taller forms were not significantly different in glyphosate content from either shorter herbs or shrubs. No significant differences were noted in AMPA by height class.

Red raspberry and oval-leaf blueberry were selected for analysis of the fruit portion of the plant due to their prevalence over the Canadian landscape and popularity as traditional-use species by multiple First Nations groups across British Columbia. These two species were grouped together for statistical analysis to ensure a representative sample size for the “berries” plant structure category. The average glyphosate level detected in berries was 142 ± 93 ppb. AMPA was not detected in any fruit.

Plants sampled were grouped by plant structure (fruit, shoot, or root) and by perennation type (WS, woody shrub; NWE, non-woody evergreen perennial; HP, herbaceous perennial) (Table 1). Statistically significant differences in glyphosate levels were detected between the shoots and roots of woody and nonwoody plants. The distributions of average glyphosate and AMPA were different across categories of perennation and plant structure (Kruskal–Wallis test, $p < 0.001$ and $p = 0.005$, respectively) (Fig. 3). According to the GLM, species and perennation-type plant structure were deemed significant factors in the glyphosate level detected (likelihood ratio $\chi^2 = 62.002$, $p < 0.001$), whereas block was not significant (Wald $\chi^2 = 7.190$, $p = 0.126$). According to GLM predicting AMPA level, only perennation-type plant structure was deemed significant (Wald $\chi^2 = 16.377$, $p < 0.001$), whereas species and block were insignificant.

The range of glyphosate values detected across all tissues analyzed were compared with the levels reported by Feng and Thompson (1990), who analyzed forest plant tissue immediately after applications, and also with the maximum residue limits (MRLs) reported as allowable by Health Canada, Canadian Food Inspection Agency (CFIA). The highest amounts detected one year after application are greater than the default amount allowed for food by the CFIA (Fig. 2) and showed much greater variability than the amounts found only 45 days after application by Feng and Thompson (1990).

Discussion

Glyphosate detection

For glyphosate to be detected in perennial shoot tissue one year after application in northern environments, glyphosate molecules first must be stored in perennating tissues through the dormant season and then translocated to new shoots and leaves when growth is initiated in the following spring. The level of glyphosate detected in shoots was highest in bunchberry, which was also the shortest plant sampled in terms of its aboveground height. Three

Fig. 2. Glyphosate (circles) and aminomethylphosphonic acid (triangles) detected in root, shoot, and berry tissues of species for all samples across all sites organized by height class. Colour band indicates the height classes of the species at maturity (blue = 5–50 cm, yellow = 50–150 cm, red = 150–300 cm); overlapping colours (observed as green and orange) indicate that height range in species can fall into more than one height class. Horizontal dotted black line marks the minimum residue limit for glyphosate set by the Canadian Food Inspection Agency.

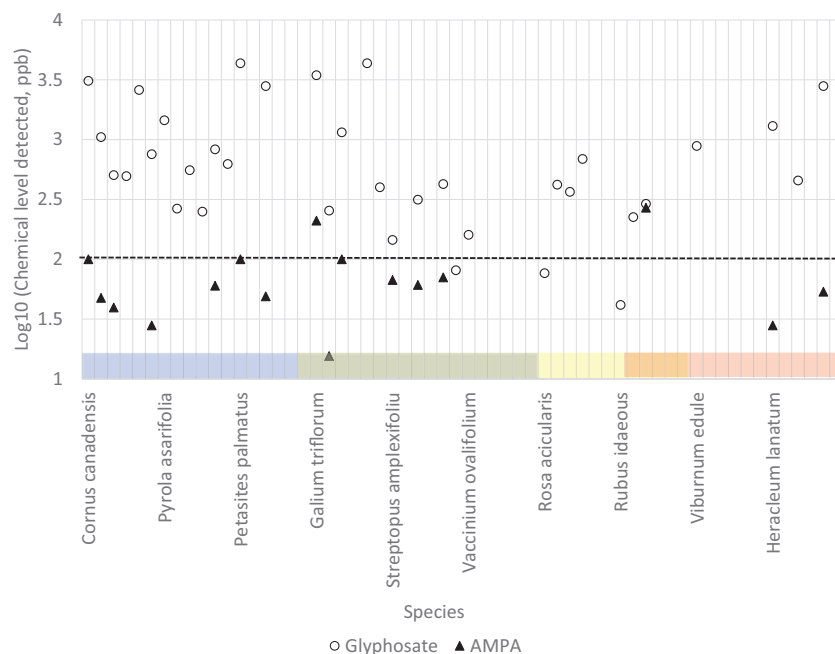
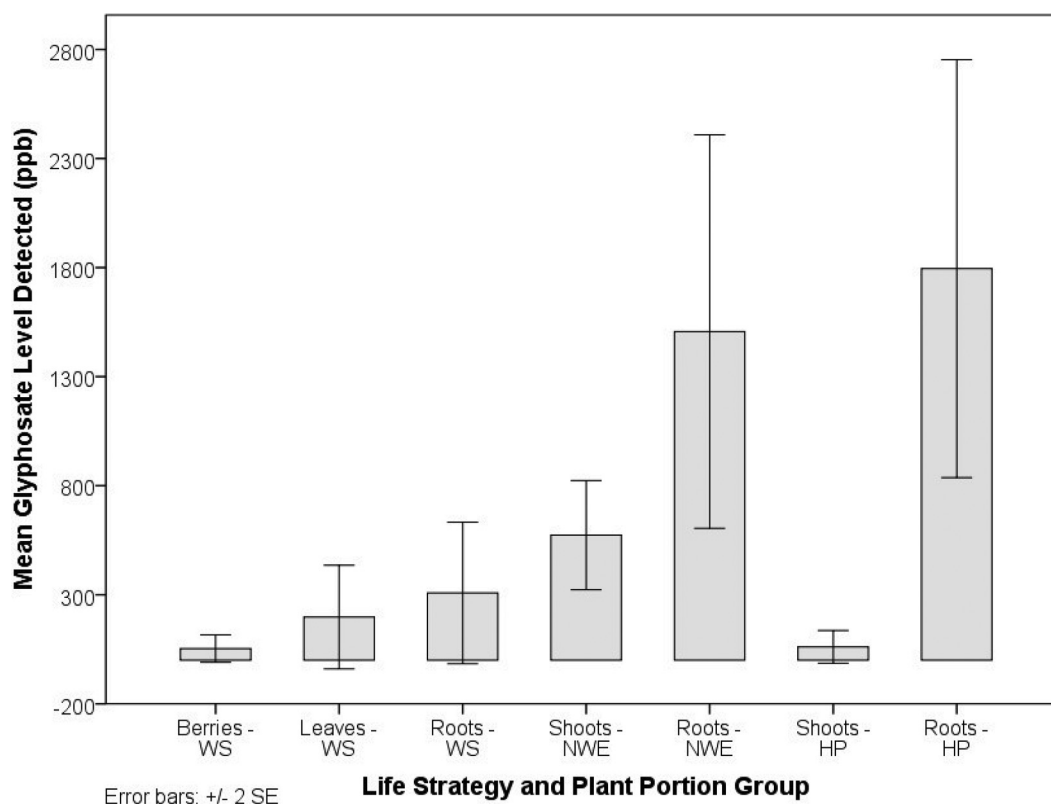


Fig. 3. Glyphosate levels (mean $\pm$ 2 SE) detected in all samples across all sample locations and years, grouped by plant structure and perennation type. WS, woody shrub; NEW, nonwoody evergreen; HP, herbaceous perennial.



of the four species in which glyphosate was detected in shoot tissue were less than 50 cm in height from the soil (bunchberry, sweet-scented bedstraw, and pink wintergreen), and plants with life forms < 50 cm showed significantly more glyphosate in tissue

than those with forms > 50 cm. It is possible that proximity to soil matrix influences the presence of glyphosate in shoot tissue in these species (Fig. 2). Glyphosate was detected more frequently in root samples than shoot samples (Table 2; Fig. 2). Glyphosate

movement mimics that of photosynthates, with primary movement occurring through phloem; therefore, it is likely that glyphosate translocation would move from source (leaves) to sink (roots) (Preston and Wakelin 2008). Because water uptake is also a main root function, it is possible that water-soluble glyphosate present in the soil matrix is absorbed by the plant roots (Wagner et al. 2003); however, given the ion-binding capacity of glyphosate in acidic soil types, it is unlikely that glyphosate is moving into plants via root systems (Miles and Moye 1988). Metal ion concentrations within tissues likely impact the presence and translocation of glyphosate within tissue given the chelating nature of glyphosate (Mertens et al. 2018).

Multiple studies discuss the response mechanisms that plants employ to tolerate glyphosate in tissue (Preston and Wakelin 2008; Rojano-Delgado et al. 2012; Sammons and Gaines 2014; Tong et al. 2017). One identified mechanism suggests that some species isolate or localize glyphosate molecules to a given tissue type leading to an ability to resist mortality (Preston and Wakelin 2008; Rojano-Delgado et al. 2012). The root structures of highbush cranberry, common cowparsnip, and palmate coltsfoot in this study were found to contain glyphosate, whereas the shoot portions tested did not (Table 2), which indicates a possible localization strategy to isolate glyphosate within roots in these species.

Glyphosate levels in plants sampled were analyzed by perennation type. Three different life strategies were demonstrated in the plants collected; these plant types store carbohydrates and other plant compounds in different ways to align with their growth strategies (Chapin et al. 1990). Significant differences in persistent glyphosate and AMPA were shown between life strategies (Fig. 3). The most “permanent” shoot tissue type, the woody shrub, contained the lowest levels of glyphosate, and the most “temporary” shoot tissue type investigated, the herbaceous perennial, contained the most glyphosate relative to other plant types. It is possible that herbaceous perennial roots have a greater storage capability for molecules such as glyphosate compared with woody shrubs, because their perennation strategy involves the fundamental necessity to store all materials in the rootstalk for use in the following year.

Two out of two blocks in which berries were sampled tested positively for glyphosate. The berries collected were fresh, thus uncontaminated by the treatment itself, and therefore, it follows that glyphosate was translocated from other plant parts to the berries in the three cases found: twice in red raspberry and once in oval-leaf blueberry. Levels detected were low; however, the presence of glyphosate in fruit one year after spraying may not be expected by managers and public due to the misconception that glyphosate degrades “quickly”. Unexpected findings may be concerning for individuals depending on their ethical stance and understanding of scientific processes. Therefore, if individuals are concerned about potential consumption of, or exposure to, glyphosate in fruit, it is recommended that they familiarize themselves with the pertinent chemical information prior to gathering and harvesting fruit in areas where glyphosate has been applied in the past (Halsall 2007; Henderson et al. 2010).

Compared with levels detected in forest plants immediately after application by Feng and Thompson (1990), levels detected in this study are very low. However, the highest levels detected in some root materials in our study, one year after application, were greater than levels reported by Feng and Thompson (1990) after only 45 days, and the levels detected were more variable. Their study site was located at a lower latitude and in a coastal climate, which could explain this difference; plant types studied had similar life strategies to the woody shrubs presented here. Levels detected in this study were above some of the specific levels allowed by CFIA for foods. Foods without a designated MRL are compared with a default allowable limit of 0.1 ppm (Health Canada 2018). The average glyphosate residue level in samples from this study, where detected, was 0.79 ppm, and the highest

level detected was greater than 4 ppm, which is well above the default allowable limit of 0.1 ppm for any nondesignated food. These levels are also above specific MRLs for plant-based foods including, for example, asparagus, which has a MRL of 0.5 ppm, as well as corn, flax seed, beans, and lentils (Health Canada 2018). Although low levels of glyphosate have been deemed nontoxic and safe for human consumption in some cases, the assessment of allowable limits is obviously plant- and source-specific (Health Canada 2018). Some people feel that any level of glyphosate contamination is unacceptable, and therefore, it becomes an ethical choice to make sure that information is available about the possible presence of glyphosate in forest plant tissues.

Aminomethylphosphonic acid detection

AMPA was found in most root structures but only two shoot samples (Fig. 2). The presence of AMPA may indicate the ability for glyphosate to be partially metabolized within tissue during the first year in which the plant tissue is exposed or that AMPA was translocated into the plants via root uptake after glyphosate degradation by soil microbes (Laitinen et al. 2006; Reddy et al. 2008) or microbial endophytes (Kryuchkova et al. 2014). To determine whether or not AMPA detected in tissue as presented here is evidence of glyphosate metabolism, genetic analysis of the species investigated is required to confirm the presence of a glyphosate oxidoreductase (GOX) gene type (Reddy et al. 2008). The location of AMPA detection (shoot or root) provided evidence of where potential metabolic activity and (or) storage within each species may have taken place. Clasp twig, red raspberry, common cowparsnip, palmate coltsfoot, and pink wintergreen only contained AMPA in the root structures despite the testing of multiple tissue types, thereby illustrating possible isolation of AMPA to rooting systems in these species. Interestingly, AMPA was only detected in nonwoody plants (Table 2). These findings provide a basis for future genetic investigation of these species.

Resource management implications

The species' investigated in this study have different ethnobotanical uses. The red–orange berries of the bunchberry plant are consumed by many First Nations, including the Dene Tsa'a Tse K'na'i people (Bannister 2006) and the Gitksan Nation (Mackinnon et al. 1999). Cooked leaves of pink wintergreen were used by the Dene Tsa'a Tse K'na'i people as a wash to treat chickenpox (Bannister 2006). Young shoots of clasp twig are used as a salad green in Alaska (Mackinnon et al. 1999). The fruit of sweet-scented bedstraw was used as a coffee substitute, and the dried flowers are used as a perfume (Mackinnon et al. 1999). The young stems of common cowparsnip were eaten directly as a food source by many First Nations (Mackinnon et al. 1999), whereas the roots were used as a poultice for rheumatism by the Carrier and Gitksan Nations (Mackinnon et al. 1999). Palmate coltsfoot was used as a cough suppressant (Mackinnon et al. 2014).

That glyphosate will persist throughout the shoot and root systems of plants for at least one year after application should be communicated to plant harvesters and users. Glyphosate may cause a disruption to the medicinal quality of plants harvested; there is evidence that glyphosate disrupts some plant secondary metabolites, and these metabolites could be the phytochemicals responsible for a given medicinal effect (Lydon and Duke 1989).

Despite the operational best practices used for the aerial application of glyphosate, namely the control of spray drift and targeted dispersal via aircraft calibration, release height, droplet size, and wind speeds, drift does still occur and off-target plant species both within and outside targeted blocks are inevitably affected by herbicide treatments (Thompson et al. 2012). The consistent detection of glyphosate in off-target plants (primarily growing underneath the canopy of targeted aspen trees) demonstrated in this study illustrates the necessity of further research into the duration of glyphosate persistence in plant tissues. Tra-

ditional plant users should take into consideration that the presence of glyphosate does not indicate toxicity; however, more research is required on long-term, low-level persistent glyphosate before any conclusion can be reached about its full direct and indirect impacts to ecosystem health. Glyphosate applicators should increase awareness of glyphosate persistence so that plant users can make informed choices about their consumption. Currently, within BC, signage is required to indicate that herbicide application has taken place on a given site. The signage recommends no entry for a 24-h period. It is recommended that the posted information be re-evaluated to include longer term information about glyphosate persistence in plant tissues.

It may be possible to develop realistic guidelines or criteria for the use of glyphosate in areas that have high value for berry-picking or plant use, allowing potential risks to plant harvesters to be minimized. The development of a tool to estimate the likelihood of glyphosate presence and translocation in plant tissue would help to guide managers in their use of glyphosate and would also provide information to the public and First Nations about their exposure to glyphosate or AMPA when harvesting plants. This assurance and transparency in operation would surely increase trust between communities and forest managers, ultimately leading to increased social license.

Acknowledgements

The author thanks Andy Carveth for seeking to initiate this research and to Don Scott for realizing the importance of continued glyphosate research in northern British Columbia. The author would also like to thank Dr. Bill McGill for his advice and contributions to revision stages of this manuscript and Spectrum Resource Group Inc. for support throughout the project. West Fraser Mills, Chetwynd Forest Industries, provided financial support for this research.

References

- Alberta Research Council. 1980. Sundance and Kenzie soil survey results. Alberta Research Council, Edmonton, Alta., Bull. No. 39.
- Al-Rajab, A.J., and Hakami, O.M. 2014. Behavior of the non-selective herbicide glyphosate in agricultural soil. *Am. J. Environ. Sci.* **10**(2): 94–101. doi:10.3844/ajessp.2014.94.101.
- Ando, C., Li, L., Walters, J., Gana, C., Segawa, R., Sava, R., and Goh, K. 2002. Residues of forestry herbicides in plants of interest to Native Americans in California national forests. EH 02-08. State of California Environmental Protection Agency, Department of Pesticide Regulation, Environmental Monitoring Branch, Sacramento, Calif.
- Bannister, K. 2006. Prophet River ethnobotany: a report on traditional plant knowledge and contemporary concerns of the Prophet River First Nation. Ravenscall Enterprises Ltd., Thetis Island, B.C.
- Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., and Séralini, G.E. 2007. Time- and dose-dependent effects of roundup on human embryonic and placental cells. *Arch. Environ. Contam. Toxicol.* **53**(1): 126–133. doi:10.1007/s00244-006-0154-8. PMID:17486286.
- Bergström, L., Börjesson, E., and Stenström, J. 2011. Laboratory and lysimeter studies of glyphosate and aminomethylphosphonic acid in a sand and a clay soil. *J. Environ. Qual.* **40**(1): 98–108. doi:10.2134/jeq2010.0179. PMID:21488498.
- British Columbia Ministry of Forests, Lands, and Natural Resource Operations. 2016. Figure “Brushing — Chemical Use (Air and Ground)” obtained through personal communication with Robert Au, Ministry of Environment, 22 June 2016.
- Canadian Council of Ministers of the Environment (CCME). 2012. Canadian water quality guidelines: glyphosate. Canadian Council of Ministers of the Environment, Winnipeg, Man., Sci. Crit. Doc. PN 1469.
- Cerdeira, A.L., and Duke, S.O. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. *J. Environ. Qual.* **35**: 1633–1658. doi:10.2134/jeq2005.0378. PMID:16899736.
- Chapin, F.S., III, Schulze, E., and Mooney, H.A. 1990. The ecology and economics of storage in plants. *Annu. Rev. Ecol. Syst.* **21**: 423–447. doi:10.1146/annurev.es.21.110190.002231.
- Cornish, P.S. 1992. Glyphosate residues in a sandy soil affect tomato transplants. *Aust. J. Exp. Agric.* **32**: 395–399. doi:10.1071/EA9920395.
- Coupe, R.H., Kalkhoff, S.J., Capel, P.D., and Gregoire, C. 2011. Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manage. Sci.* **68**: 16–30. doi:10.1002/ps.2212.
- DeLong, C., Banner, A., MacKenzie, W.H., Rogers, B.J., and Kaytor, B. 2011. A field guide to ecosystem identification for the Boreal White and Black Spruce Zone of British Columbia. British Columbia Ministry of Forests and Range, Forest Science Program, Victoria, B.C., Land Manage. Handb. 65.
- Duke, S.O. 2011. Glyphosate degradation in glyphosate-resistant and -susceptible crops and weeds. *J. Agric. Food Chem.* **59**(11): 5835–5841. doi:10.1021/jf102704x. PMID:20919737.
- Duke, S.O., and Powles, S.B. 2008. Glyphosate: a once-in-a-century herbicide. *Pest Manage. Sci.* **64**(4): 319–325. doi:10.1002/ps.1518.
- Duke, S.O., Lydon, J., Koskinen, W.C., Moorman, T.B., Chaney, R.L., and Hammerschmidt, R. 2012. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *J. Agric. Food Chem.* **60**: 10375–10397. doi:10.1021/jf302436u. PMID:23013354.
- Environment Canada. 2017. Past weather and climate, historical data for Dawson Creek station number 1182289 [online]. Available from http://climate.weather.gc.ca/historical_data/search_historic_data_e.html.
- Farstad, L., Lord, T.M., Green, A.J., and Hortie, H.J. 1965. Soil survey of the Peace River area in British Columbia. Report No. 8 of the British Columbia Soil Survey. University of British Columbia and Canada Department of Agriculture Research Station, Vancouver, B.C.
- Feng, J.C., and Thompson, D.G. 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. *J. Agric. Food Chem.* **38**(4): 1118–1125. doi:10.1021/jf00094a046.
- Government of British Columbia. 2017. BC Soil Information Finder Tool. Available from <http://www2.gov.bc.ca/gov/content/environment/air-land-water/land/soil-information-finder> [accessed 17 April 2017].
- Govindarajulu, P.P. 2008. Literature review of impacts of glyphosate herbicide on amphibians: what risks can the silvicultural use of this herbicide pose for amphibians in British Columbia? British Columbia Ministry of Environment, Victoria, B.C., Wildlife Report No. R-28.
- Hadi, F., Salmanian, A.H., Mousavi, A., Ghazizadeh, E., Amani, J., and Noghabi, K.A. 2012. Development of quantitative competitive PCR for determination of copy number and expression level of the synthetic glyphosate oxidoreductase gene in transgenic canola plants. *Electron. J. Biotechnol.* **15**(4): 2.
- Halsall, C.J. 2007. Section 6.4. Chemical partitioning. In *Principles of environmental chemistry*. Edited by R.M. Harrison. Royal Society of Chemistry, Cambridge, U.K. doi:10.1039/9781847557780-00279.
- Health Canada. 2018. Consumer product safety: maximum residue levels database [online]. Available from <http://pr-rp.hc-sc.gc.ca/mrl-lrm/results-eng.php> [accessed 30 July 2018].
- Helander, M., Saloniemi, I., and Saikkonen, K. 2012. Glyphosate in northern ecosystems. *Trends Plant Sci.* **17**: 569–574. doi:10.1016/j.tplants.2012.05.008. PMID:22677798.
- Henderson, A., Gervais, J., Luukinen, B., Buhl, K., and Stone, D. 2010. Glyphosate technical fact sheet [online]. Oregon State University Extension Services, National Pesticide Information Center. Available from <http://npic.orst.edu/factsheets/glyphotech.pdf>.
- Howe, A.R., Gasser, C.C., Brown, S.M., Padgett, S.R., Hart, J., Parker, G.B., Fromm, M.E., and Armstrong, C.L. 2002. Glyphosate as a selective agent for the production of fertile transgenic maize plants. *Molecular Breeding*, **10**(3): 153–164.
- Kissane, Z., and Shephard, J.M. 2017. The rise of glyphosate and new opportunities for biosentinel early-warning studies. *Conserv. Biol.* **31**(6): 1293–1300. doi:10.1111/cobi.12955. PMID:28474816.
- Kremer, R., Means, N., and Kim, S. 2005. Glyphosate affects soybean root exudation and rhizosphere micro-organisms. *Int. J. Environ. Anal. Chem.* **85**: 1165–1174. doi:10.1080/03067310500273146.
- Kryuchkova, Y.V., Burygin, G.L., Gogoleva, N.E., Gogolev, Y.V., Chernyshova, M.P., Makarov, O.E., Fedorov, E.E., and Turkovskaya, O.V. 2014. Isolation and characterization of a glyphosate-degrading rhizosphere strain, *Enterobacter cloacae* K7. *Microbiol. Res.* **169**: 99–105. doi:10.1016/j.micres.2013.03.002. PMID:23545355.
- Laitinen, P., Siimes, K., Eronen, L., Rämö, S., Welling, L., Oinonen, S., Mattsoff, L., and Ruohonen-Lehto, M. 2006. Fate of the herbicides glyphosate, glufosinate-ammonium, phenmedipham, ethofumesate and metamitron in two Finnish arable soils. *Pest Manage. Sci.* **62**: 473–491. doi:10.1002/ps.1186.
- Lord, T., Green, A., and Hennig, A. 1977. A soils and land use tour of the Peace River lowland. Unpublished report. Agriculture Canada, Vancouver, B.C.
- Lydon, J., and Duke, S.O. 1989. Pesticide effects on secondary metabolism of higher plants. *Pestic. Sci.* **25**(4): 361–373. doi:10.1002/ps.2780250406.
- Mackinnon, A., Pojar, J., and Coupe, R. 1999. Plants of Northern British Columbia. Lone Pine Publishing, Vancouver, B.C.
- Mackinnon, A., Kershaw, L., Arnason, J., Owen, P., Karst, A., and Hamersley Chambers, F. 2014. Edible and medicinal plants of Canada. Lone Pine Publishing, Edmonton, Alta.
- Mertens, M., Höss, S., Neumann, G., Afzal, J., and Reichenbecher, W. 2018. Glyphosate, a chelating agent relevant for ecological risk assessment? *Environ. Sci. Pollut. Res. Int.* **25**(6): 5298–5317. doi:10.1007/s11356-017-1080-1.
- Miles, C.J., and Moye, H.A. 1988. Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils. *J. Agric. Food Chem.* **36**: 486–491. doi:10.1021/jf00081a020.
- Monsanto Canada Inc. 2011. VisionMAX™ Silviculture Herbicide commercial solution label [online]. Monsanto, Winnipeg, Man. Available from https://www.for.gov.bc.ca/ftp/toc/external/publish/Integrated_Pest_Management_plan/Glyphosate/Vision%20Max/Vision%20Max%20Label.pdf.
- Preston, C., and Wakelin, A.M. 2008. Resistance to glyphosate from altered

- herbicide translocation patterns. *Pest Manage. Sci.* **64**: 372–376. doi:[10.1002/ps.1489](https://doi.org/10.1002/ps.1489).
- Reddy, K.N., Rimando, A.M., Duke, S.O., and Nandula, V.K. 2008. Aminomethylphosphonic acid accumulation in plant species treated with glyphosate. *J. Agric. Food Chem.* **56**: 2125–2130. doi:[10.1021/jf072954f](https://doi.org/10.1021/jf072954f). PMID:[18298069](https://pubmed.ncbi.nlm.nih.gov/18298069/).
- Relyea, R.A. 2005. The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecol. Appl.* **15**: 1118–1124. doi:[10.1890/04-1291](https://doi.org/10.1890/04-1291).
- Rojano-Delgado, A.M., Cruz-Hipolito, H., de Prado, R., de Castro, M.D.L., and Franco, A.R. 2012. Limited uptake, translocation and enhanced metabolic degradation contribute to glyphosate tolerance in *Mucuna pruriens* var. *utilis* plants. *Phytochemistry*, **73**: 34–41. doi:[10.1016/j.phytochem.2011.09.007](https://doi.org/10.1016/j.phytochem.2011.09.007).
- Sammons, R.D., and Gaines, T.A. 2014. Glyphosate resistance: state of knowledge. *Pest Manage. Sci.* **70**: 1367–1377. doi:[10.1002/ps.3743](https://doi.org/10.1002/ps.3743).
- Schrübers, L.C., Masís-Mora, N., Carazo Rojas, E., Valverde, B.E., Christensen, J.H., and Cedergreen, N. 2016. Analysis of glyphosate and aminomethylphosphonic acid in leaves from *Coffea arabica* using high performance liquid chromatography with quadrupole mass spectrometry detection. *Talanta*, **146**: 609–620. doi:[10.1016/j.talanta.2015.07.059](https://doi.org/10.1016/j.talanta.2015.07.059). PMID:[26695310](https://pubmed.ncbi.nlm.nih.gov/26695310/).
- Sharma, S.D., and Singh, M. 2001. Environmental factors affecting absorption and bio-efficacy of glyphosate in Florida beggarweed (*Desmodium tortuosum*). *Crop Prot.* **20**: 511–516. doi:[10.1016/S0261-2194\(01\)00065-5](https://doi.org/10.1016/S0261-2194(01)00065-5).
- Stenrød, M., Eklo, O.M., Charnay, M.P., and Benoit, P. 2005. Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils. *Pest Manage. Sci.* **61**: 887–898. doi:[10.1002/ps.1107](https://doi.org/10.1002/ps.1107).
- Thompson, D., Leach, J., Noel, M., Odsen, S., and Mihajlovich, M. 2012. Aerial forest herbicide application: comparative assessment of risk mitigation strategies in Canada. *For. Chron.* **88**(2): 176–184. doi:[10.5558/tfc2012-034](https://doi.org/10.5558/tfc2012-034).
- Tong, M., Gao, W., Jiao, W., Zhou, J., Li, Y., He, L., and Hou, R. 2017. Uptake, translocation, metabolism, and distribution of glyphosate in nontarget tea plant (*Camellia sinensis* L.). *J. Agric. Food Chem.* **65**: 7638–7646. doi:[10.1021/acs.jafc.7b02474](https://doi.org/10.1021/acs.jafc.7b02474).
- Turner, N. 2010. *Plants of Haida Gwaii*. Sono Nis Press, Winlaw, B.C.
- Wagner, R., Kogan, M., and Parada, A.M. 2003. Phytotoxic activity of root absorbed glyphosate in corn seedlings (*Zea mays* L.). *Weed Biol. Manage.* **3**: 228–232. doi:[10.1046/j.1444-6162.2003.00110.x](https://doi.org/10.1046/j.1444-6162.2003.00110.x).
- Wiersema, R., Burns, M., and Hershberger, D. 1997. Biocatalysts/biodegradation database: glyphosate pathway map [online]. University of Minnesota. Available from http://umbbd.ethz.ch/gly/gly_map.html [accessed 29 January 2014].
- Woodburn, A.T. 2000. Glyphosate: production, pricing and use worldwide. *Pest Manage. Sci.* **56**: 309–312. doi:[10.1002/\(SICI\)1526-4998\(200004\)56:4<309::AID-PS143>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<309::AID-PS143>3.0.CO;2-C).