Forest Ecology and Management 493 (2021) 119259

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Glyphosate remains in forest plant tissues for a decade or more

N. Botten [a], L.J. Wood [b,*], J.R. Werner [c,d]

[a] University of Northern British Columbia, Natural Resource and Environment Studies, Canada
[b] University of Northern British Columbia, Faculty of Environment, Canada
[c] British Columbia Ministry of Forests, Lands, and Natural Resource Operations and Rural Development, Prince George, BC, Canada
[d] University of Northern British Columbia, Ecosystem Science and Management, Canada

ARTICLE INFO

Keywords:
Glyphosate
Sub-lethal herbicide
Persistence
AMPA
Forest ecology

ABSTRACT

Glyphosate-based herbicides are highly effective, non-selective, and broad-spectrum herbicides that have been used in British Columbia's forest industry since the early 1980's. Over this time, long-term persistence of glyphosate has not been measured, largely due to the inability to analyze glyphosate at low concentrations. Given the advancements in analytical techniques that are now available, we have extended the persistence curve of glyphosate to elucidate the actual length of time of persistence in northern British Columbia, rather than relying on estimations of persistence based on half-life curves that are quite often modelled from incomparable environments. We collected plant tissues from five forest understory perennial species growing in two distinct biogeoclimatic regions of northern BC to map out how glyphosate residue quantities change over time according to species, plant tissue type, and climate regime. We found that residues persisted for up to 12 years in some tissue types, and that root tissues generally retained glyphosate residues longer than shoot tissue types. We also found that samples from the colder, more northern biogeoclimatic zone investigated retained significantly higher levels of glyphosate for longer than samples collected from the warmer biogeoclimatic zone.

1. Introduction

Glyphosate (N-(phosphonomethyl) glycine) is the most widely used herbicide in the world, in both agricultural and forestry industries, as well as for invasive weed control and household yard and garden use (Henderson et al. 2010). A highly effective, non-selective, broad-spectrum herbicide first introduced in 1974, it is present as the active ingredient in numerous glyphosate-based herbicides (GBHs), including the Roundup®, Vision®, and VisionMax® formulations manufactured by Monsanto Company (Baylis 2000; Dost 2003; Thompson & Pitt 2011). Innumerable studies on glyphosate, especially in the form of Roundup®, have been published in relation to its agricultural use. In comparison, there is a deficiency of research available focusing on the Vision® group of formulas and their use in forestry.

Upon application, glyphosate is absorbed through leaves, stems or roots (Bernards et al. 2005), and is translocated throughout the plant. This translocation follows the source to sink flow of photosynthates (sucrose and other carbohydrates) through the phloem, and after cycling throughout the plant for at least 72 h, glyphosate accumulates in the apical meristems of roots and young leaves (Fadin et al. 2018; Machado et al. 2009; Bernards et al. 2005). Glyphosate can be released to the surrounding soil by plant roots, where it may be strongly adsorbed to soil particles, degraded by microorganisms, or absorbed by adjacent plant roots (Viti et al. 2019).

Once inside plant tissues, glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme required for the biosynthetic shikimic acid pathway that produces the amino acids tyrosine, phenyl alanine and tryptophan (Duke et al. 2012; Richmond 2018). These amino acids are vital to protein synthesis and plant growth; thus, disruption of the shikimic acid pathway by glyphosate effectively kills the plant (Henderson et al. 2010; Richmond 2018). The enzyme EPSPS is present in plants and microorganisms, but not in animal cells (OECD 1999). For this reason, it is widely believed that glyphosate is harmless to humans and animals (Dost 2003; Duke et al. 2012). However, there continues to be much debate and controversy about the safety of glyphosate (Landrigan & Belpoggi 2018; Richmond 2018; Larsson et al. 2018; Zhang et al. 2019).

Glyphosate is degraded in the soil through metabolization by microorganisms, a complex process, the rate of which depends upon multiple factors, including the type of microbe, soil pH, moisture, temperature, and other climatic variables (Helander et al. 2012). In northern climates, prolonged freezing of the soil during winter months may

* Corresponding author.at: Faculty of Environment, University of Northern British Columbia, 3333 University Way, Prince George, BC V2N4Z9, Canada.
E-mail address: Lisa.wood@unbc.ca (L.J. Wood).

https://doi.org/10.1016/j.foreco.2021.119259
Received 5 March 2021; Received in revised form 7 April 2021; Accepted 9 April 2021

reduce the rate of glyphosate degradation by microbial action (Stenrød et al. 2005), though it is possible that microorganisms may adapt somewhat to subfreezing soils (Newton et al. 2008). The primary metabolite of glyphosate is aminomethylphosphonic acid (AMPA), and both glyphosate and AMPA negatively impact plant physiology (Gomes et al. 2014). Presence of AMPA in plant tissues may be due to absorption from the soil (Gomes et al. 2014), or may be evidence of degradation of glyphosate within the plant (Tong et al. 2017). The effects of cold climate on the degradation of glyphosate within plant tissues are unknown.

In Canada, glyphosate has been used on over 90% of herbicide-treated forest areas nationwide (Thompson & Pitt 2011). The province of Ontario accounts for over 40% of glyphosate use in Canada, with British Columbia (BC) ranking second at 17% (Govindarajulu 2008). Silvicultural applications of glyphosate account for approximately 34% of total glyphosate use (by weight sold) in BC, with the majority being used for agriculture and horticulture (Govindarajulu 2008). In BC, approximately 17,000 ha/year of forested land has been sprayed with herbicides (primarily GBH) since 1985, largely for conifer release (Government of British Columbia 2016).

When herbicides are sprayed on forest cutblocks aerially, it is difficult to predict the exact dosage that any individual plant will receive (Feng & Thompson 1990). The concentrations reaching understory plants growing closer to the forest floor, such as small herbs and shrubs, are affected by overtopping vegetation structure and height, wind, precipitation, and overlap as the aircraft makes multiple passes over the cutblock (Lloyd 1990). In both forestry and agriculture, there is also a risk of GBH reaching non-targeted species in adjacent areas through spray drift or overspray (Boutin et al. 2014; Cederlund 2017), or through runoff (Govindarajulu 2008). Further, in a forest ecosystem, the targeted species rarely grow isolated from other plant species, and many non-targeted plants are sprayed with GBH simply due to their proximity to the targeted species, often at a sub-lethal dose as a result of being located in the understory (Wood 2019). Conversely, a targeted species may also receive a sub-lethal dose due to incomplete coverage.

The effects of low concentrations of spray drift on non-targeted plants are complex and not well-understood (Cederlund 2017). Due to variable levels of sensitivity to glyphosate, some non-targeted plants die, and surviving plants may translocate and store glyphosate within their tissues (Florencia et al. 2017; Székács & Darvas 2012). Glyphosate and AMPA may persist in perennial plant tissues for an extended duration of time of a year or more (Roy et al. 1989; Mamy et al. 2016; Wood 2019). These plants may experience deformities, growth suppression and other negative effects, even though the concentration reaching non-targeted plants in this situation is typically very low (Timms & Wood 2020; Florencia et al. 2017). The exact duration of residue persistence is unknown for plants in forested environments of British Columbia. Many of these non-targeted plants are foraged upon by various wildlife species, and some are also wild-harvested by humans for consumption or medicinal usage. The value of these plants may be questionable if they contain glyphosate.

Very little research has been conducted on glyphosate storage and persistence within plant tissues, and to our knowledge, no research has yet been conducted on long-term glyphosate persistence in perennial forest plants beyond one year after treatment. Most of the existing data on this topic refer to glyphosate content in the tissues of harvested food crop species, particularly glyphosate-resistant crops (example: Bøhn et al. 2014), or in forest plants immediately after GBH application (Roy et al. 1989; Feng & Thompson 1990). The perennial nature of the majority of forest plants, combined with a growing awareness of adverse effects of chronic, low-doses of glyphosate on health and the environment, indicates that more research should be conducted regarding the long-term effects of glyphosate in a forested environment. Further research is required to determine the duration of glyphosate persistence in plant tissues, particularly in a forestry context. The aim of this research project was to determine the duration (from one year up to twelve years) of glyphosate persistence in selected perennial forest plant tissues, and to compare residue levels within roots, shoots, and fruits. Presence of AMPA was also evaluated.

2. Methods

2.1. Study areas

The Province of BC maintains a Biogeoclimatic Ecosystem Classification (BEC) system, which delineates the 900,000 + km^2 province into fourteen ecological zones and numerous subzones based on differences and variation in climate, soils and vegetation (Meidinger & Pojar 1991). Our sampling sites were chosen from two different BEC zones within the interior of BC: the Boreal White and Black Spruce (BWBS) zone and the Sub-Boreal Spruce (SBS) zone (Fig. 1). The BWBS zone extends across Canada, and on a global scale, is part of the circumpolar boreal zone (DeLong et al. 2011). It features a northern continental climate with frequent exposure to arctic air masses, short growing seasons, and long, very cold winters during which the ground freezes deeply (Meidinger & Pojar 1991). This zone is generally colder and drier than adjacent zones in the winter, and can be warmer in the summer (DeLong et al. 2011). The SBS zone is a montane zone that dominates BC's central interior and adjoins the BWBS zone to the north. The SBS zone features a continental climate with seasonal extremes of temperature: severe, snowy winters, moderate annual precipitation, and relatively warm, moist, and short summers (Meidinger & Pojar 1991). The sub-boreal climate of the SBS zone is slightly warmer in January and cooler in July, and has shorter winters and a slightly longer growing season than the more continental boreal climate of the BWBS zone (Meidinger & Pojar 1991). Table 1 provides a comparison of some climatic features and the dominant tree species in each zone.

The forest cutblocks sampled had all been clearcut logged prior to GBH treatment. The cutblocks were planted 1-4 years after logging and the GBH treatments were applied 3–5 years post-plant as a means of controlling the competing aspen (*Populus tremuloides*) stems. As a result, the vegetation sampled for this study were considered largely "non-target", and were likely exposed to varying concentrations of GBH as droplets fell through the young, inconsistent aspen canopies.

2.2. Experimental Design & sampling

Samples of roots and shoots were collected from four species of plants chosen for their importance to the diet of moose, bears and other wildlife, their importance to local traditional plant users, and to represent various plant growth strategies: *Salix* spp. (willow), *Cornus sericea* L., syn. *C. stolonifera* (red-osier dogwood), *Rubus idaeus* L. (red raspberry), and *Chamaenerion angustifolium* (L.) Scop. (fireweed). Fruits were also collected from *R. idaeus* and *Vaccinium caespitosum* Michx. (dwarf blueberry) plants, both of which are commonly eaten by humans and wildlife.

Fireweed is an herbaceous perennial with rhizome-like roots and 0.5–3 m tall stems, and is especially common in disturbed areas and open forests (MacKinnon et al. 1999). Despite its prolific wind-borne seed distribution, fireweed reproduces primarily by sending up new shoots each spring from buds that formed late in the previous growing season along a complex horizontal root system that may survive for decades (Broderick 1990). It is considered "an early to mid-successional invader of the boreal forest," with the ability to colonize from seed in disturbed sites and quickly dominate, persisting in later successional stages (Pinno et al. 2013). Fireweed is consumed by wildlife, including both moose (Broderick 1990) and bears (Ciarniello 2018).

Red-osier dogwood is a stoloniferous shrub (meaning that it has horizontal stems, or stolons, at the soil surface) 1–4 m tall, growing in moist soils. It is an extremely important winter food source for moose (Zach et al. 2011), and the berries are an important food for bears in Northern British Columbia (Noyce & Garshelis 2011; Benson &

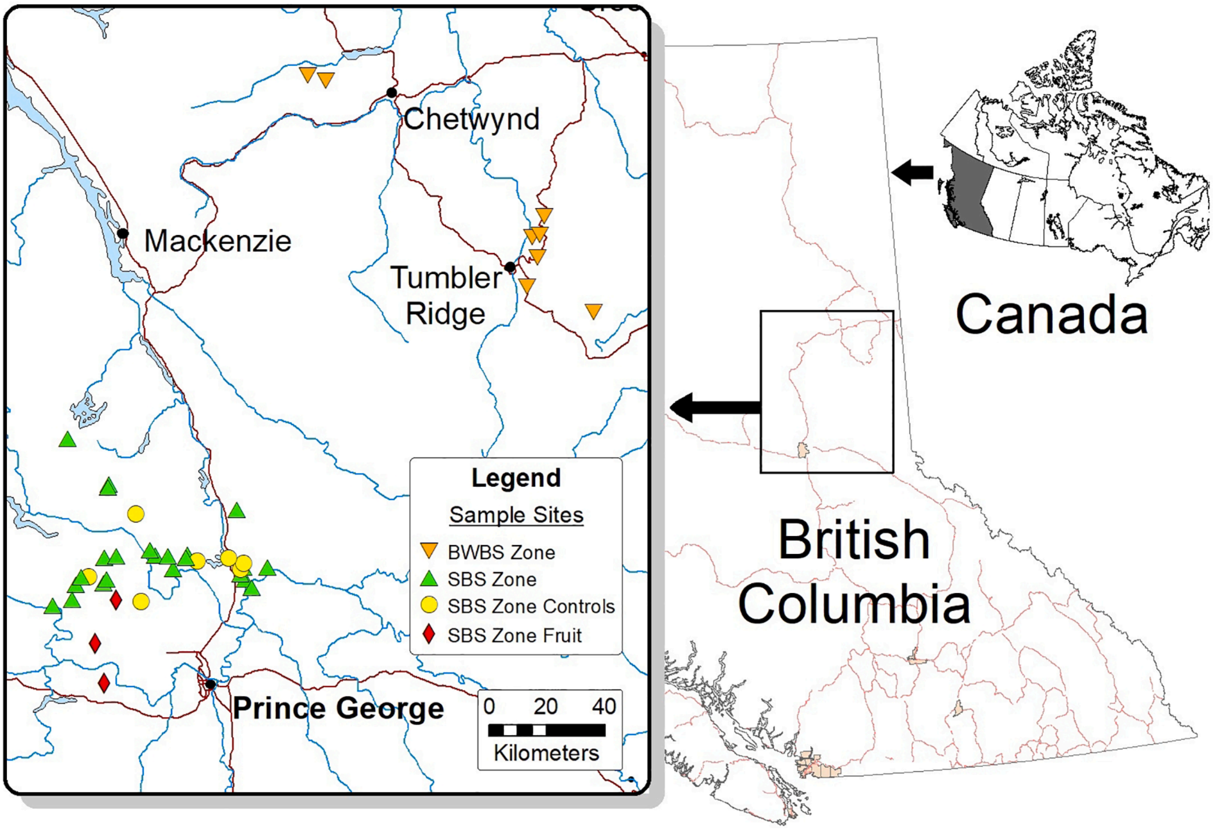

Fig. 1. Study sites: Samples were collected from forest cutblocks in British Columbia, Canada, within the Sub-Boreal Spruce (SBS) Biogeoclimatic zone near the city of Prince George, and within the Boreal White and Black Spruce (BWBS) zone near the towns of Tumbler Ridge and Chetwynd.

Table 1
Comparison of Boreal White and Black Spruce (BWBS) and Sub-Boreal Spruce (SBS) Biogeoclimatic Ecosystem Classification (BEC) zones (adapted from Meidinger & Pojar 1991). [a] – Updated with 1971–2000 climate normals (DeLong et al. 2011). The BWBS zone has a colder climate with longer winters and less precipitation than the SBS zone.

	BWBS	SBS
Mean annual temperature [a]	1.7 (range −2.4 °C to 3.6) °C	2.2 (range 0.7 °C to 4.2) °C
Months with average temperature < 0 °C	5–7	4–5
Months with average temperature > 10 °C	2–3	3–5
Mean temp, coldest month	−24.5 °C to −17.7 °C	−14.6 °C to −7.7 °C
Mean temp, warmest month	12.0 °C to 16.6 °C	12.9 °C to 16.9 °C
Mean annual precipitation [a]	525 (range 341–897) mm	708 (range 436–1893) mm
Proportion of annual precipitation falling as snow	35–55%	25–50%
Mean annual snowfall	135–269 cm	111–379 cm
Major tree species	White spruce, trembling aspen, lodgepole pine, black spruce, balsam poplar, tamarack, subalpine fir, common paper birch, Alaska paper birch	Climax species: hybrid white spruce, subalpine fir, black spruce. Seral species: lodgepole pine, trembling aspen, paper birch, douglas-fir.

Chamberlain 2006).

Willows are known for being difficult to identify to the species level (MacKinnon et al. 1999), and commonly form hybrid subspecies. For this reason, we have not identified the species, but collected samples from a variety of available *Salix* spp. shrubs. Willow is a staple diet item for moose and other herbivores, and is also important for bedding and cover (MacKinnon et al. 1999).

Red raspberry is a perennial shrub, up to 1.5 m tall with upright stems (canes), typically found in low to moderate elevation habitats that have been disturbed by logging, silvicultural operations, or fire (MacKinnon et al. 1999). Bearing biennial canes from a perennial root system, which produce fruit in their second year, red raspberry is a pioneer invader that rapidly develops an extensive root system and foliage to colonize recently disturbed open forest areas, surviving for many years afterward (Oleskevich et al. 1996). Although initial colonization is generally via seed germination, and abundant quantities of seeds are produced thereafter, red raspberry spreads primarily via vegetative reproduction once established, through short-lived root suckers from extensive clonal colonies (Oleskevich et al. 1996). Raspberry fruits and foliage are eaten by both wildlife (Oleskevich et al. 1996; Ciarniello 2018) and people, and the leaves are used medicinally (MacKinnon et al. 1999).

Dwarf blueberry is a perennial deciduous shrub that grows up to 0.3 m high and can be found throughout northern BC (MacKinnon et al. 1999). Blueberries are eaten by wildlife, including bears (Ciarniello 2018), and people (MacKinnon et al. 1999).

Root and shoot samples were collected in July of 2018 on forestry cutblocks where VisionMax® glyphosate-based herbicide was aerially applied at a rate of 3.3–4.0 L/ha (resulting in a concentration of 1.78–2.16 kg a.i./ha), one year, three years, six years, and twelve years before sample collection (corresponding to the treatment years 2017, 2015, 2012, and 2006), following standard forestry operational procedures for aerial herbicide application. In each region, for each application year, composite samples were collected for each species and tissue type, from each of ten plots in treated areas. In the BWBS zone, corresponding control samples were collected from ten plots in untreated areas within the same cutblocks (Fig. 2). In the SBS zone, control samples were collected from separate cutblocks of the same age (logged in the same year). Plots were each a minimum of 100 m away from any other plot and 20 m from the edge of the treatment zone. Separate samples of roots and shoots were collected for each species. Each composite sample contained tissues from a minimum of three individual plants of the same species, collected using pruning shears, treeplanting spades, and trowels. Unless absent, one sample of each tissue type of each plant species was collected in each plot, resulting in up to ten treated sample replicates and ten control sample replicates of each type for each spray year in each BEC zone (fewer control samples were collected in the SBS zone). The ten sample replicates of each type were collected over at least two different cutblocks per exposure year to ensure a genetically diverse sample selection. Plant samples were frozen in sealed plastic bags until they were processed.

Fruit samples were collected in August of 2019 on forestry cutblocks in the SBS BEC zone where VisionMax GBH was applied aerially at a rate of 3.3 L/ha (resulting in a concentration of 1.78 kg a.i./ha), one year prior (i.e. sprayed in 2018). Sampling was done in the same manner as described above, resulting in the collection of nineteen treated and six control raspberry fruit samples from three different cutblocks, and ten treated and ten control blueberry fruit samples from one cutblock. A further nine treated and four control samples of raspberry fruits were picked off of shoot samples that were collected in July of 2018 from sites treated six years before sample collection (in 2012).

2.3. Sample processing and laboratory analysis

Plant samples were individually washed with a minimum of three rinses to remove all traces of soil (with the exception of raspberry fruit samples collected in 2019, which were too juicy to wash and lacked visible soil particles), dried, ground to a powder, and returned to the freezer until they could be sent to the lab for chemical analysis. Root and shoot samples were dried at 80 °C, and fruit samples were dried at 60 °C, in a Lindberg / Blue Gravity Oven (Model # GO1330SA). Grinding was accomplished with the following, depending on tissue type and mill availability: Thomas Wiley Mini Mill (T4276M) with a 40 mesh (0.425 mm) screen; IKA A 11 basic Analytical mill; Kinematica POLYMIX ® PX-MFC 90 D with a 0.8 mm or 0.5 mm screen; Hamilton Beach Custom Grind coffee grinder (80393C) or Cuisinart Grind Central coffee grinder (PG-13658FA-CAN), each with a removeable washable stainless steel grinding bowl; and a mortar & pestle. Grinders were blown clean with forced air between similar samples from the same spray year, and washed with soap and water and dried between samples of different types or spray years.

Samples were analyzed for presence of glyphosate and AMPA by the University of Guelph Agriculture & Food Laboratory, using high performance liquid chromatography – mass spectrometry (HPLC-MS). Since costs for residue analysis were high, we selected priority sample groups for chemical analysis (Table 2).

Table 2
Plant species & tissue types analyzed by the University of Guelph Agriculture & Food Laboratory, using high performance liquid chromatography – mass spectrometry (HPLC-MS), for glyphosate and aminomethylphosphonic acid residues sampled from two different biogeoclimatic zones in forests of northern British Columbia, Canada.

Species	Tissue Types: Sub-Boreal Spruce Zone	Tissue Types: Boreal White & Black Spruce Zone
C. angustifolium (fireweed)	Shoot: 9 controls; 44 treated Root: 12 controls; 44 treated	Shoot: 35 controls; 40 treated Root: 35 controls; 39 treated
Salix spp. (willow)	Shoot: 13 controls; 40 treated	Shoot: 32 controls; 38 treated
C. sericea (red osier dogwood)	Shoot: 10 controls; 46 treated	–
R. idaeus (red raspberry)	Fruit: 10 controls; 28 treated	Shoot: 24 controls; 27 treated Root: 26 controls; 21 treated
V. caespitosum (dwarf blueberry)	Fruit: 10 controls; 10 treated	–

Willow, dogwood, and fireweed shoots are key components in the diet of moose, and were chosen specifically for this reason, though they are also consumed by other herbivores and omnivores. Raspberry and blueberry fruits were chosen because they are commonly consumed by humans, bears, and other wildlife. Although they may also be foraged upon or have ethnobotanical uses, fireweed and raspberry roots, as well as raspberry shoots, were chosen primarily to provide a means of comparing residue allocation between different plant tissues. A total of 377 treated and 216 control (untreated) samples were analyzed.

2.4. Statistical analysis

The residue data received from the laboratory (Wood et al. 2021) included numerical values where the amount detected was > 0.03 ppm, and non-numeric values including: "not detected"; "<MDL" = Less than the minimum detection limit of 0.008 ppm; and "<MQL" = Less than the minimum quantification limit of 0.03 ppm. The < MDL and < MQL categories indicate a confirmed presence of the compound (whether glyphosate or AMPA) by HPLC-MS but at less than the routine detection limit, or the defined quantification limit, respectively. To include these qualitative results as detected numeric quantities in the analyses, "not detected" was given a value of zero, and we substituted the average between the minimum and maximum possible concentrations for < MDL and < MQL. Concentrations of < MDL were taken as 0.004 ppm (median between 0.000 and 0.008) and concentrations of < MQL were taken as 0.019 ppm (median between 0.008 and 0.03) (Wood et al. 2021).

With a high proportion of zero values resulting from samples with no detected residues, the glyphosate and AMPA concentration data errors were strongly skewed to the right, and thus did not satisfy the assumption of normality, as confirmed by Shapiro-Wilk tests.

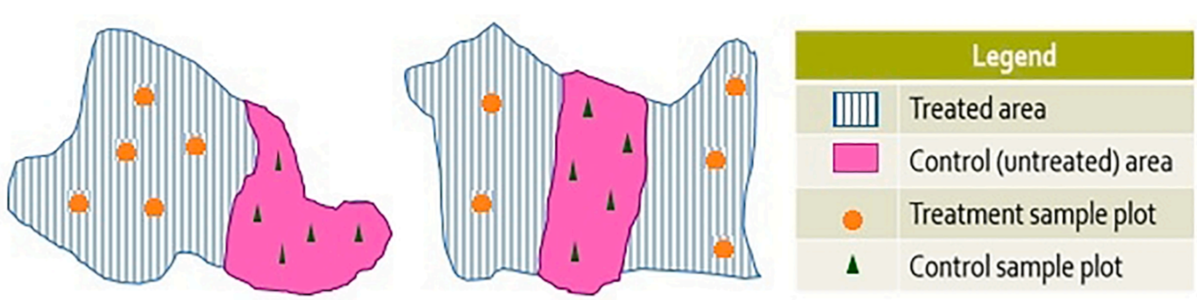

Fig. 2. Sampling Design – Examples of sampling plot layouts in forest cutblocks in northern British Columbia, Canada, where cutblocks were composed of both glyphosate-based herbicide treatment areas and untreated areas (stratified for specific management at the time of treatment).

Accordingly, Kruskal-Wallis H tests (analysis of variance by ranks for non-parametric data) were conducted, using Stata Statistical Software 14.2 (StataCorp LLC 2018), to determine the effects of the independent categorical variables ("year", "BEC zone", "species", and "tissue type") on glyphosate and AMPA concentrations, and are reported using the standard $\chi 2$ with degrees of freedom in parentheses. We compared only same tissue types between species (i.e. shoot to shoot, root to root, or fruit to fruit) because of the differing storage capabilities of plant tissue types. Significant test results were followed by post hoc multiple pairwise comparisons between groups, using Dunn's Test with a Sidak adjustment for multiple comparisons. Throughout, an α of 0.05 was used to assess significance.

Generalized Linear Models (GLMs) were run in IBM SPSS Statistics 26.0 software (IBM Corp. 2019) to determine the value of each independent categorical variable as a model predictor for both concentration and presence of glyphosate and AMPA in plant tissues. GLMs with a Tweedie distribution and a log link were used to determine the value of the independent variables as predictors for residue concentrations. The two dependent variables, glyphosate concentration and AMPA concentration, were considered as covariates. Next, binary variables were created from the residue concentration data, to indicate whether or not glyphosate and AMPA were detected in each sample. With the binary variables, GLMs with a binomial distribution and a logit link were used to determine the value of each independent variable as a predictor for residue presence.

3. Results

The 216 control samples collected from untreated areas were expected to be free from glyphosate and AMPA residues, yet 5.5% of control samples contained trace amounts of either glyphosate (seven samples, or 3.2% of the total) or AMPA (five samples, or 2.3% of the total) (Table 3). These samples, both roots and shoots, were all among those collected from untreated areas within treated cutblocks in the BWBS BEC zone. In contrast, over all years, 45% of the total 377 treated samples contained residue, with 167 (or 44%) containing glyphosate, and 69 (or 18%) containing AMPA (Table 3). All but one of the treated samples containing AMPA also contained glyphosate, for a total of 68 (18%) of treated samples containing both residues. Statistical comparison of residue concentrations showed significant differences between control and treated samples for both glyphosate ($\chi^2(1) = 111.998$, $p < 0.001$) and AMPA ($\chi^2(1) = 31.974$, $p < 0.001$) concentrations.

Table 3
Total number of combined root, shoot and fruit samples containing glyphosate and aminomethylphosphonic acid (AMPA) residues from managed forest cutblocks in northern British Columbia, Canada, for each treatment year investigated (*ypt = years post-treatment with glyphosate-based herbicide).

		Glyphosate		Aminomethylphosphonic Acid (AMPA)	
	n	Detected	% Detected	Detected	% Detected
Control Samples					
1 ypt*	71	0	0.00%	1	1.41%
3 ypt	39	5	12.82%	4	10.26%
6 ypt	61	1	1.64%	0	0.00%
12 ypt	37	1	2.70%	0	0.00%
All Years Combined	216	7	3.24%	5	2.31%
Treated Samples					
1 ypt	118	110	93.22%	60	50.85%
3 ypt	76	34	44.74%	7	9.21%
6 ypt	100	21	21.00%	1	1.00%
12 ypt	83	2	2.41%	1	1.20%
All Years Combined	377	167	44.30%	69	18.30%

3.1. Residue persistence over time and by biogeoclimatic zone

Glyphosate and AMPA were significantly reduced over time, in terms of both presence and concentration, in all plant tissues ($p < 0.001$) (Table 3). The proportion of samples containing glyphosate decreased exponentially from 93% to 2% over the twelve-year period. Over the same time period, the proportion of samples containing AMPA decreased to 1% at 12 years after treatment, approximating a logarithmic decline.

Glyphosate and AMPA both remained in plant tissues for a longer duration in the BWBS zone compared with the SBS zone. The rate of decrease, expressed as the proportion of detections, varied between the zones, with the proportion of samples with detected glyphosate much higher at years three and six in the BWBS zone compared with the same years in the SBS zone (Fig. 3). No AMPA was detected in any samples from the SBS zone at three or more years after treatment. The trend is nearly identical whether considering only the sample types that were collected from both BEC zones (fireweed roots and shoots, and willow shoots), or when all sampled species are plotted together, including those that were sampled from only one BEC zone (Fig. 3).

Generalized linear models validated the trend shown in Fig. 3, indicating that samples from the BWBS zone have a greater likelihood of containing detectable amounts of glyphosate ($p < 0.001$) and AMPA ($p < 0.001$), as well as higher concentrations of both residues ($p < 0.001$ for each), compared with samples from the SBS zone. The difference in residue concentrations, however, was not always statistically significant when looking at individual treatment years, species, or tissue types.

Kruskal-Wallis tests confirmed that there were significant differences in both glyphosate ($\chi^2(1) = 14.668$, $p < 0.001$) and AMPA ($\chi^2(1) = 11.219$, $p < 0.001$) concentrations between the two BEC zones, when all samples were considered together. The difference in residue concentration across BEC zones remained significant for all species combined when considering only samples taken at one and three years after treatment, as well as through year six for glyphosate; however, there is no significant difference in either residue type across BEC zones after twelve years, nor for AMPA concentration after six years.

3.2. Plant species and part-tissue type

Residues dropped below detection limits within the timeframe of this study for some, but not all, sample types. Glyphosate was not detected in any shoots, fruit, nor in raspberry roots, by year twelve. There was no detectable AMPA in any shoots, nor in raspberry roots by three years after treatment (Fig. 4). Both glyphosate and AMPA were detected in fireweed roots up to twelve years after treatment in two out of 20 samples (Fig. 4). Generalized linear modelling (of all samples together) showed that species, except for dogwood ($p = 0.499$), and tissue type were significant predictors of glyphosate presence ($p = 0.042$; $p = 0.012$), while only tissue type was a significant predictor of AMPA presence ($p < 0.001$). Fruit, however, was not found to be a significant predictor of glyphosate presence ($p = 0.541$) or concentration ($p = 0.185$). Similar to residue presence, species and tissue type were found, through GLMs, to be significant predictors for glyphosate concentration ($p < 0.001$; $p < 0.001$), but not for AMPA concentration. However, glyphosate and AMPA residue concentrations between species and tissue type were only significantly different in the first year after treatment.

Excluding fruit samples, at one year after treatment: only three samples did not contain glyphosate; eleven samples contained glyphosate at concentrations < MQL; ten samples contained glyphosate at concentrations < MDL; and the remaining 21 root and 44 shoot samples contained glyphosate ranging in concentration from 0.033 to 1.800 μg g^{-1}, plus a 6.500 μg g^{-1} outlier.

Roots of both raspberry and fireweed consistently contained more glyphosate than shoots did, and raspberry fruits contained the least. This difference, however, was only statistically significant for fireweed in the first year after treatment ($\chi^2(1) = 4.136$, $p < 0.041$; roots ($n = 21$) and

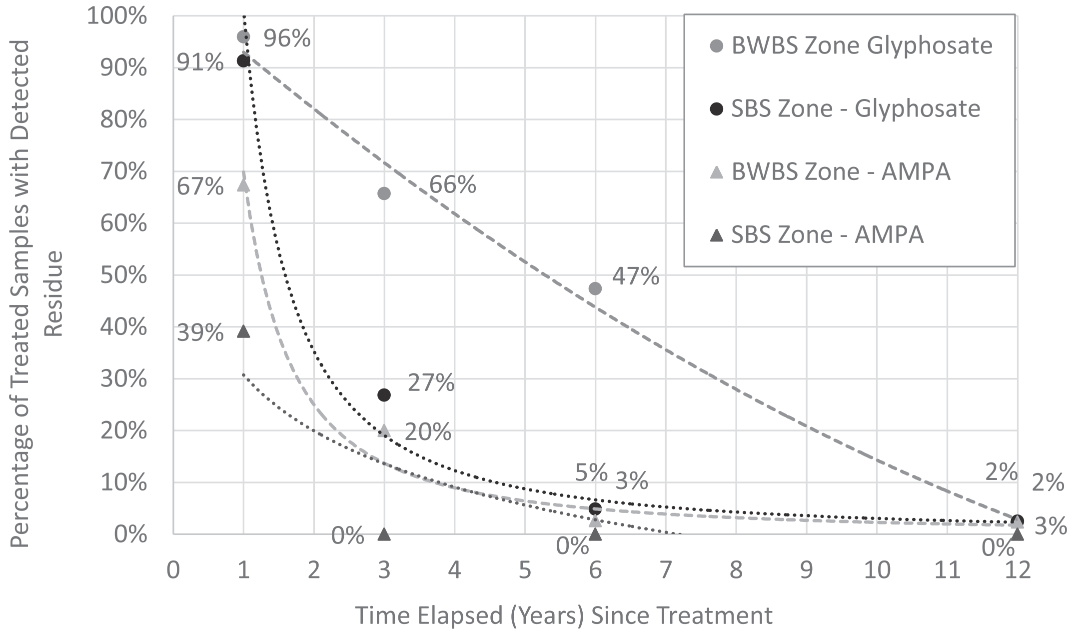

Fig. 3. Proportion of samples treated with glyphosate-based herbicides with detected glyphosate and aminomethylphosphonic acid (AMPA) residues, by time and biogeoclimatic (BEC) zone, out of those sampled from managed forests of northern British Columbia.

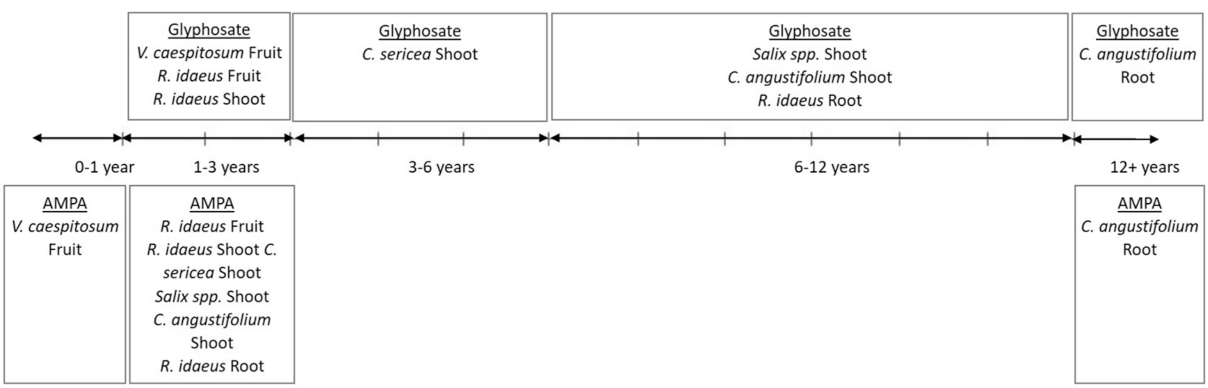

Fig. 4. Timeline of glyphosate residue persistence in native plant species and individual tissues of those species, after treatment with glyphosate based herbicides in forests of northern British Columbia, Canada. Species include: blueberry (*Vaccinium caespitosum*), raspberry (*Rubus idaeus*), fireweed (*Chamaenerion angustifolium*), red osier dogwood (*Cornus stolonifera*), and willow (*Salix spp.*). Each sample type is labeled at the approximate point in time at which residue concentrations dropped below detection limits. Some fireweed root samples still contained trace amounts of glyphosate and aminomethylphosphonic acid (AMPA) at 12 years post-treatment.

shoots (n = 19)). In the first year after treatment in particular, both fireweed and raspberry roots contained relatively high concentrations of glyphosate, with averages greater than 0.4 μg g^{-1} (0.467 μg g^{-1} and 0.437 μg g^{-1} respectively). The difference in glyphosate concentration between years one and three was not significant for fireweed roots, and there was significantly more glyphosate in fireweed and willow samples after three years than at twelve years post-treatment (Fig. 6). Glyphosate concentrations > MQL ranged from 0.037 to 0.33 μg g^{-1} after six years, and one fireweed root sample contained 0.17 μg g^{-1} of glyphosate twelve years after treatment. Two fireweed root samples collected at one year after treatment contained the greatest concentrations of glyphosate in this study, at 1.800 μg g^{-1}, and 6.500 μg g^{-1} (an exceptionally high value relative to other values in this study). The concentrations of both residues were determined to be statistically different between the roots of fireweed and raspberry ($\chi^2(1) = 6.481$, $p = 0.011$ and $\chi^2(1) = 8.154$, $p = 0.004$, respectively). Fireweed roots were the only sample type that contained any residue at twelve years post-treatment, and the only ones that contained AMPA at six years post-treatment (Fig. 6). Fireweed roots contained statistically more AMPA than the shoot portion of the plants sampled, after one year ($\chi^2(1) = 15.111$, $p < 0.001$) and three years ($\chi^2(2) = 7.417$, $p = 0.007$), but not after six years ($\chi^2(2) = 1.286$, $p = 0.257$), or twelve years ($\chi^2(2) = 0.950$, $p = 0.330$), when concentrations were very low in both shoots and roots.

In the first year after treatment, 100% of dogwood, raspberry, and willow shoot samples and 89% of fireweed shoot samples contained glyphosate, and the highest concentrations of glyphosate residue in shoots were found in dogwood, followed by willow, and raspberry. Fireweed shoots had the lowest concentrations of glyphosate compared to the other species, and the only statistically significant difference in quantity at one year after treatment ($\chi^2(3) = 30.743$, $p < 0.001$) (Figs. 5 & 6). Raspberry shoots contained no glyphosate at year three and beyond, and dogwood shoots had no glyphosate by year six. It should be noted that the raspberry shoots in years one, three, and six are represented by a low sample number (n = 3–4) and more statistical verification in future studies may be warranted, although the decline in samples containing glyphosate follows a similar trendline as the other species. All four species were devoid of glyphosate in their shoots by twelve years post-treatment.

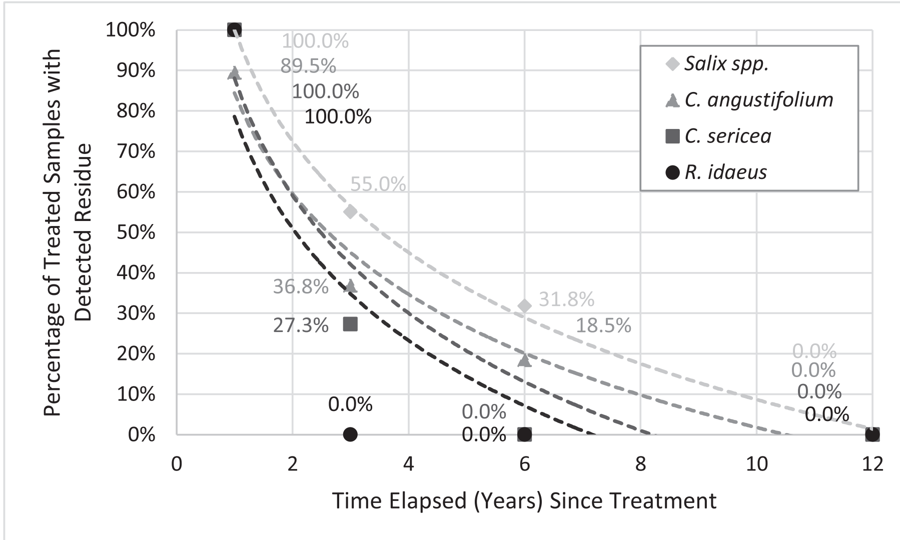

Fig. 5. Proportion of glyphosate-based herbicide-treated shoot samples with detected glyphosate residue, by species. Glyphosate was present in all samples except for some *C. angustifolium* samples at 1 year post-treatment.

Raspberry shoots contained the most AMPA, followed by willow, dogwood, and fireweed (Fig. 6). AMPA concentrations in fireweed shoots were significantly lower than in raspberry shoots ($p = 0.001$) and willow shoots ($p = 0.013$), and AMPA in dogwood shoots was significantly lower than raspberry ($p = 0.020$) one year post-treatment. There were no significant differences in the concentrations of either residue between the shoots of any species at three, six, or twelve years post-treatment (Fig. 6).

Generalized linear models predicted the significant decrease in residue concentrations for all species and tissue types over time ($\chi^2(3) = 213.708$, $p < 0.001$) (Fig. 6). In most cases, Kruskal-Wallis tests showed that the concentrations of both glyphosate and AMPA in samples collected one year after treatment were significantly greater than in samples collected three, six, or twelve years after treatment.

In fruit samples collected from the SBS BEC zone one year after treatment, a greater number of raspberries were detected with residue than blueberries: 90% of raspberries ($n = 19$) and 70% of blueberries ($n = 10$) contained glyphosate, and 68% of raspberries and none of the blueberries contained AMPA. Raspberry fruit samples had an average glyphosate residue concentration that was ten times greater than blueberry fruit samples (0.074 μg g^{-1} compared to 0.007 μg g^{-1} for blueberries) ($\chi^2(1) = 9.064$, $p = 0.002$), and significantly greater AMPA as well ($\chi^2(1) = 10.970$, $p < 0.001$). Of the thirteen raspberry fruit samples, in which glyphosate residue was detected at levels > MQL, the average glyphosate concentration was 0.105 μg g^{-1}, ranging from 0.057 to 0.21 μg g^{-1}, and five of these samples (26%) contained glyphosate at concentrations greater than the maximum residue limit (MRL) of 0.1 μg g^{-1}, set by the Government of Canada for foods (Health Canada 2012, Kolakowski et al. 2020). Only in the first year after treatment were raspberry fruits ($n = 19$) found to have a significantly lower concentration of glyphosate than either roots ($n = 10$) or shoots ($n = 10$) had ($\chi^2(2) = 20.654$, $p < 0.001$). All glyphosate concentrations in blueberry fruit were < MQL.

4. Discussion

It is widely claimed that glyphosate does not remain in the environment for any significant period of time (Newton et al. 1994; Duke 2010). Contrary to this belief, this study clearly demonstrated that surviving plants in forest cutblocks treated with GBH may contain glyphosate residue in their roots, shoots and fruits for the first full year or more after treatment, and many also contain AMPA, with some plants retaining these residues for twelve years or more. Previous research on perennial forest plants has primarily considered only short-term (much less than one year) persistence of glyphosate (or AMPA) in plant tissues. Wood (2019) showed that glyphosate ranging in concentration from 0.077 to 1.050 μg g^{-1} could be detected in the tissues of non-targeted perennial forest plants at one year after operational treatment with GBH. Prior to this, Newton et al. (1994) reported 0.162 μg g^{-1} glyphosate residue remaining in herbaceous vegetation 346 days after treating the canopy with a high dose of glyphosate. Newton et al. (1994) concluded that, since 96% of initial residues had dissipated to “levels below any known herbicidal activity” within 30 days at most sites, and because it is commonly believed that glyphosate poses no risk of toxicity, the low concentrations remaining after a year were inconsequential.

Persistent residue concentrations detected in our study were larger than some previously reported, perhaps due to improved methods of detection. For example, in fruit, where detected after one year, we found an average of approximately 0.105 μg g^{-1}, ranging from 0.057 to 0.21 μg g^{-1} using a method of low-detection HPLC-MS, where Roy et al. (1989) reported concentrations of 1.23 ± 0.248 μg g^{-1} and 1.22 ± 0.122 μg g^{-1} in fruits sampled at 33 days after treatment with GBH, and 0.19 ± 0.035 μg g^{-1} after 61 days using a GC–MS method. Keeping in mind that the concentrations reported by Roy et al. (1989) were from fruit that was sprayed directly, it is interesting that we recorded similar concentrations after one year in fruit that could only have acquired glyphosate through translocation from other tissue types. This illustrates the importance of continually revisiting policies based on science using techniques such as chemical analysis, where significant advancements in laboratory processes have evolved over the last few decades.

The concentrations of glyphosate we found present, in non-targeted plant tissues one year after treatment (0.033 to 6.500 μg g^{-1}), and even some concentrations recorded at three years after treatment, are similar to concentrations reported by these previous studies. The levels detected are also greater than the default MRL of 0.1 μg g^{-1} used by the Canadian Food Inspection Agency (CFIA) to assess foods destined for human consumption (Kolakowski et al. 2020). It may therefore be asked whether these concentrations are considered safe for wildlife to consume, especially considering that large areas of forested land are cleared and treated with GBH every year. Moose have been observed to preferentially browse in cutblocks 7–11 years after treatment with GBH, probably since the conditions at that time include a favourable combination of forage and conifers for bedding and cover (Eschholz et al.

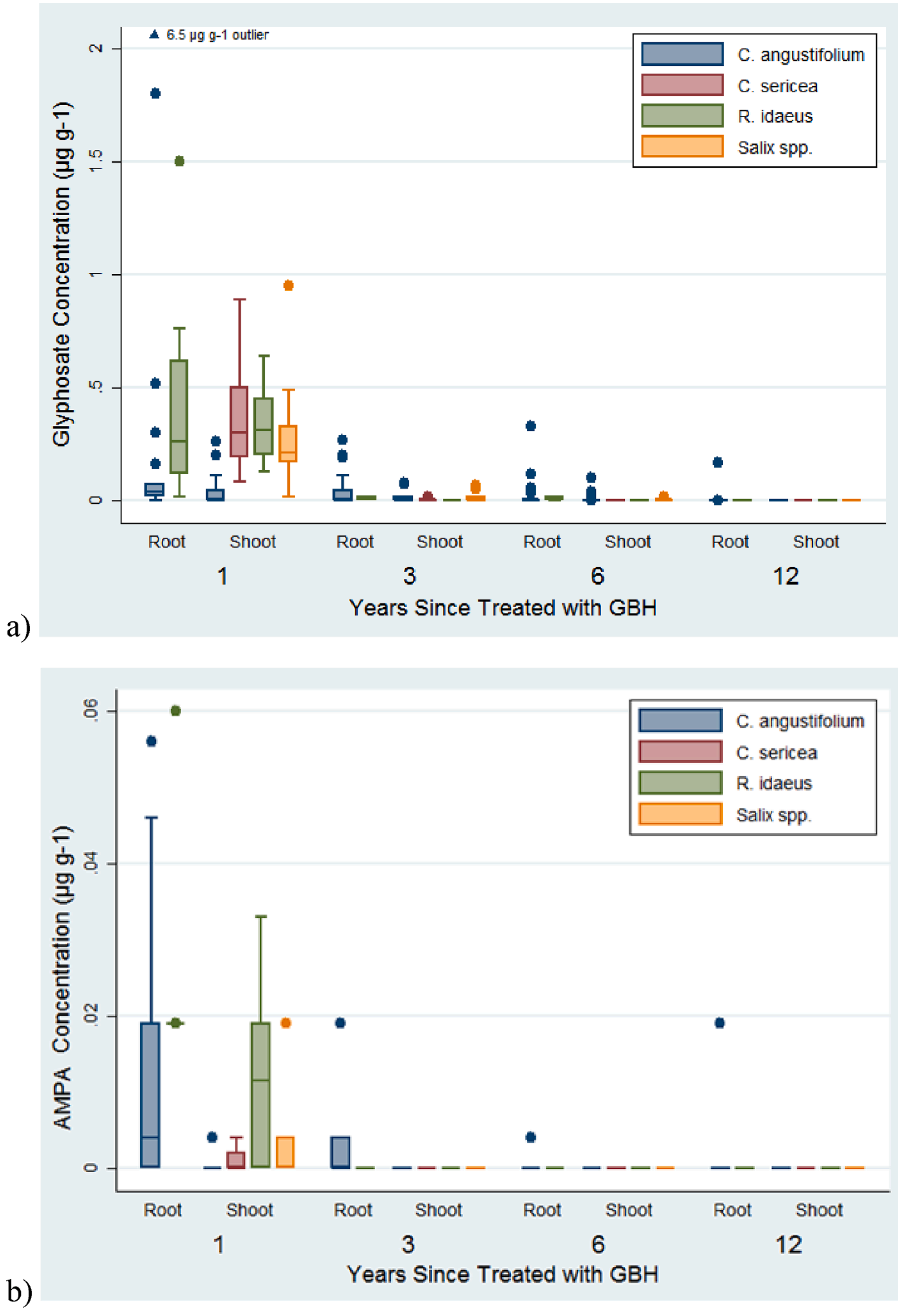

Fig. 6. Change in glyphosate and aminomethylphosphonic acid (AMPA) residue concentrations over time in plant tissues (roots and shoots) treated with glyphosate-based herbicides in forests of northern British Columbia, Canada. a) Glyphosate by species & part, b) AMPA by species & part. Note that y-axes are different scales.

1996). Whether persistent glyphosate in plant tissues in these areas might have an effect on the health of moose and other wildlife species is not known.

Trace amounts of glyphosate have been documented in soil, air, water, and food (Landrigan & Belpoggi 2018); exposure to sub-lethal concentrations of glyphosate is likely to be chronic for both humans and wildlife, especially in urban and agricultural areas, and a growing body of research has linked such exposure to various negative health effects (Kissane and Shephard 2017; Barnett and Gibson 2020). Little information is available on long-term health effects of chronic exposure to glyphosate (Richmond 2018), but it can be expected that wildlife exposed directly to glyphosate during or shortly after application, as well as to low concentrations of glyphosate residue in their foods, may be at greater risk of developing chronic health problems (Barnett and Gibson 2020). However, limited research has been conducted regarding the effects of glyphosate on wildlife (Kissane and Shephard 2017), and due to divided factions regarding whether or not glyphosate is toxic (Zyoud et al. 2017), it is difficult to determine the extent of such effects at this time.

The persistence of glyphosate and AMPA within perennial forest plant tissues is a source previously unaccounted for, and the knowledge that these residues remain in plant tissues for much longer than previously suspected, even at very low concentrations, must be considered by forest professionals when making vegetation management decisions. Further, whether or not glyphosate and its metabolic products are considered harmful to flora or fauna at low concentrations, any compound deliberately added to the environment by humans should be accounted for appropriately. That some control samples unexpectedly contained residues further highlights the fact that even at very low application rates such as those experienced by understory plants through spray drift, trace amounts of glyphosate and AMPA may be stored within plant tissues for twelve years or more.

As expected, AMPA was detected less frequently and in lower quantities than glyphosate. It also decreased over time more rapidly than did glyphosate, however, the fact that plant tissues contained any amount of AMPA may indicate metabolic breakdown of glyphosate within the plant tissues (Tong et al. 2017), a phenomenon that is not well understood. It is known that some plants have a gene for a microbial enzyme (GOX) that converts glyphosate to AMPA, which has been used in genetically engineered plants to make them resistant to glyphosate (Heap and Duke 2018). It is possible that another mechanism for glyphosate degradation within plants exists.

The clear differences in both presence and concentrations of glyphosate and AMPA in samples from the two different BEC zones are likely a result of differences in climate regime. Degradation of glyphosate in plant tissues may be affected by duration of plant dormancy, which is in turn associated with climatic conditions. The more northern BWBS zone has a colder climate than the SBS zone has, with slightly longer winters and 5–7 months with average temperatures below freezing, compared with only 4–5 months below freezing in the SBS. This difference in climate regime undoubtedly affects the rate of glyphosate and AMPA decomposition in the soil, since microbial activity is reduced under freezing conditions (Newton et al. 2008). Increased duration of persistence of glyphosate in soil in colder climates could play a role in the quantities of residues observed in plant tissues if reuptake by roots occurs (Tong et al. 2017). Glyphosate can reach the soil directly, during GBH application; through exudation by plant roots (Viti et al. 2019); as well as through leaves shed by contaminated plants (Mamy et al. 2016), whether due to seasonal defoliation or die-off as a result of herbicidal action (Newton et al. 1994). Prior research has suggested that glyphosate applied to soil is strongly bound and very slow to leach regardless of soil type (Al-Rajab and Hakami 2014). Therefore, any movement of glyphosate from soil to plant or plant to soil would more likely be attributed to differences in plant species physiology rather than the soil type.

It might be expected that individual species of plants will demonstrate unique tolerances to and storage capacities for glyphosate and AMPA (Florencia et al. 2017), however, the differences in concentrations found in tissues were mostly insignificant, especially when compared with the effects of BEC zone (Fig. 3). The only differences found between glyphosate and AMPA concentrations across species, were at one year after treatment. At this point, residues were significantly lower in shoots of fireweed, an herbaceous perennial, compared with shoots of the other species, all woody perennials. This finding could be because the entire shoot of an herbaceous perennial plant dies off annually, while woody species retain their stems. The shoots of herbaceous plants analyzed in this study (fireweed) were never directly in contact with the GBH that was applied a year or more before sampling occurred, so any glyphosate present in the shoots of herbaceous plants must therefore have been translocated from roots. Woody plant shoots, in contrast, may contain glyphosate and AMPA residues that were stored in shoots since the original application, as well as residues that were translocated back into shoots from the roots. Wood (2019) suggested that the strategy of herbaceous perennials to store all resources in the root over winter may result in a greater storage capacity in roots for molecules such as glyphosate, which was reflected by the roots of herbaceous perennial plants containing the highest concentrations of glyphosate and AMPA. Our present results are consistent with this finding, but only if mean concentrations are considered rather than median: in this case, the herbaceous fireweed roots have slightly greater concentrations than the woody raspberry roots have, as a result of a few fireweed root samples containing far greater concentrations than the majority of other samples. However, the differences between raspberry and fireweed roots are not that simple, as raspberry roots actually had a greater median concentration. Further study would be of benefit.

Furthermore, we observe that across the four species tested for shoot residues, the frequency of occurrence varies by species, but residues in all species follow a similar rate of degradation over the 12-year period (Fig. 5). However, the trend shown in Fig. 5 is nearly identical whether considering only the species that were sampled in both BEC zones (fireweed and willow), or when all sampled species are plotted together, including those that were sampled from only one BEC zone. This similarity indicates that BEC zone has more of an influence on the presence of glyphosate and AMPA residue in plant tissues over time than has species.

The other significant difference found between species at one year after treatment was between blueberry and raspberry fruits: raspberry fruits had greater incidence and concentration of glyphosate and AMPA than did blueberry fruits. Plant height could be a contributing factor to this difference; *V. caespitosum* is a very low shrub, up to 0.3 m high, while *R. ideaus* grows up to 1.5 m tall (MacKinnon et al. 1999), although the canes in this study were generally under 1 m in height. It is conceivable that the taller raspberry plants received a higher dose of GBH than did the potentially more sheltered blueberry plants. Leaf size may be another factor: raspberry leaves are much larger than blueberry leaves, possibly resulting in more interception of spray (Timms & Wood 2020). It is also plausible that the two species have different storage capacities for glyphosate.

Fruits clearly contained the lowest residue concentrations in this study, and roots contained the highest concentrations overall. Once absorbed by plant foliage, glyphosate moves from source to sink, accumulating primarily in the roots. In addition, since perennial plants shed their leaves annually, and the stems of herbaceous perennials also die off in the winter, some of the residue stored in the leaves (and stems of herbaceous plants) is lost to leaf litter. Although fruit contained the least residue of all tissue types on average, 26% of fruit samples contained concentrations greater than the 0.1 μg g^{-1} MLR used by the Canadian Food Inspection Agency to assess glyphosate residue content in foods. These 26% of fruit samples would be deemed unfit for human consumption if assessed in the marketplace. Residual glyphosate in fruits in the year following treatment with GBH could have chronic implications for wildlife such as birds, bears, and other mammals consuming large quantities of berries in forest cutblocks.

5. Conclusions

Glyphosate, when applied at sub-lethal doses, such as that experienced by plants in the understory and in adjacent areas during standard applications of GBH in forest cutblocks, persists in plant tissues for a minimum of one year after treatment, and in some cases still remains in trace amounts after twelve or more years. The quantities of glyphosate contained in plant tissues after 3–12 years are extremely low, and should not be considered an immediate hazard, however, the cumulative effects of long-term residual glyphosate should be considered when assessing exposure of humans and wildlife to chronic, low-concentrations of glyphosate and other chemicals in the environment.

Climatic conditions can impact the duration of glyphosate persistence in plant tissues, as indicated by the clear differences in both glyphosate and AMPA concentrations in plant tissues from two different BEC zones. This should be considered by forest managers, especially in the more northern boreal forests of Canada, where glyphosate may persist in both soils and plant tissues for longer than previously expected.

Roots retain more glyphosate than do shoots, and they retain it for a longer duration. Although many shoot samples contained glyphosate at one year post treatment, no shoot samples contained glyphosate at three or more years after treatment. Herbaceous and woody perennial plants may have differing abilities to store glyphosate and AMPA, though more research is required before a definitive statement can be made on this subject. AMPA detected within plant tissues may indicate metabolization of glyphosate within plant tissues.

Although residue concentrations in fruits were lower than those in root and shoot tissues, both raspberry and blueberry fruits contained low quantities of glyphosate in fresh growth at one year after treatment, some of which were above the MRL for human consumption. Further research on glyphosate and AMPA content in edible portions of plants at 1–5 years after treatment with GBH would be beneficial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was conducted with the financial support of the National Science and Engineering Research Council of Canada (NSERC), British Columbia Timber Sales, West Fraser Mills Ltd – Chetwynd Forest Industries, and Habitat Conservation Trust Foundation; and the in-kind support of the British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development. The authors would like to also thank Spectrum Resource Group Inc. for their in-kind support of this research. The funding organizations were not involved in the study design; in the collection, analysis and interpretation of data; or in the writing of the report.

References

Al-Rajab, A.J., Hakami, O.K., 2014. Behaviour of the non-selective herbicide glyphosate in agricultural soil. Am. J. Environ. Sci. 10 (2), 94–101.

Barnett, J., Gibson, D., 2020. Separating the empirical wheat from the pseudoscientific chaff: A critical review of the literature surrounding glyphosate, dysbiosis and wheat-sensitivity. Front. Microbiol. 11, 556729. doi: 10.3389/fmicb.2020.556729.

Baylis, A.D., 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Manag. Sci. 56, 299–308. https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<299::AID-PS144>3.0.CO;2-K.

Benson, J.F., Chamberlain, M.J., 2006. Food habits of Louisiana Black Bears (*Ursus americanus luteolus*) in two subpopulations of the Tensas River basin. Am. Midland Nat. 156, 118–127.

Bernards, M.L., Thelen, K.D., Penner, D., Muthukumaran, R.B., McCracken, J.L., 2005. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Sci. 53, 787–794. https://doi.org/10.1614/WS-05-043R.1.

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, R., 2014. Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chem. 153, 207–215. https://doi.org/10.1016/j.foodchem.2013.12.054.

Boutin, C., Strandberg, B., Carpenter, D., Mathiassen, S.K., Thomas, P.J., 2014. Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications? Environ. Pollut. 185, 295–306. https://doi.org/10.1016/j.envpol.2013.10.009.

Broderick, D.H., 1990. The Biology of Canadian Weeds.: 93. *Epilobium angustifolium* L. (Onagraceae). Can. J. Plant Sci. 70, 247–259. https://doi.org/10.4141/cjps90-027.

Cederlund, H., 2017. Effects of spray drift of glyphosate on nontarget terrestrial plants—A critical review. Environ. Toxicol. Chem. 36, 2879–2886. https://doi.org/10.1002/etc.3925.

Ciarniello, L., 2018. A Review of Food Security for Grizzly Bears in British Columbia. Technical report. The Grizzly Bear Foundation, Vancouver, BC. Available online: www.researchgate.net/publication/323737695_A_Review_of_Food_Security_for_Grizzly_Bears_in_British_Columbia (accessed 1 January 2021).

DeLong, C., British Columbia, Ministry of Forests and Range, 2011. A field guide to ecosystem identification for the boreal white and black spruce zone of British Columbia. B.C. Ministry of Forests and Range, Victoria, BC. Available online: https://www.for.gov.bc.ca/hfd/pubs/Docs/Lmh/Lmh65.htm (accessed 1 January 2021).

Dost, F.N., 2003. Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options: Part IV, Risk to workers using glyphosate formulations. Forest Practices Branch, BC Ministry of Forests, Victoria. URL: http://www.llbc.leg.bc.ca/public/pubdocs/bcdocs/363001/6_dost_glyphosate.pdf (accessed 2 February 2019).

Duke, S. 2010. Glyphosate Degradation in Glyphosate-Resistant and –Susceptible Crops and Weeds. J. Agric. Food Chem. 2011, 59, 5835–5841. dx.doi.org/10.1021/jf102704x |.

Duke, S.O., Lydon, J., Koskinen, W.C., Moorman, T.B., Chaney, R.L., 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. https://doi.org/10.1021/jf302436u.

Eschholz, W.E., Servello, F.A., Griffith, B., Raymond, K.S., Krohn, W.B., 1996. Winter Use of Glyphosate-Treated Clearcuts by Moose in Maine. J. Wildl. Manag. 60, 764–769. https://doi.org/10.2307/3802375.

Fadin, D.A., Tornisielo, V.L., Barroso, A.A.M., Ramos, S., Dos Reis, F.C., Monquero, P.A., Kudsk, P., 2018. Absorption and translocation of glyphosate in Spermacoce verticillata and alternative herbicide control. Weed Res. 58, 389–396. https://doi.org/10.1111/wre.12329.

Feng, J.C., Thompson, D.G., 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. J. Agric. Food Chem. 38, 1118–1125. https://doi.org/10.1021/jf00094a046.

Florencia, F.M., Carolina, T., Enzo, B., Leonardo, G., 2017. Effects of the herbicide glyphosate on non-target plant native species from Chaco forest (Argentina). Ecotoxicol. Environ. Saf. 144, 360–368. https://doi.org/10.1016/j.ecoenv.2017.06.049.

Gomes, M.P., Smedbol, E., Chalifour, A., Hénault-Ethier, L., Labrecque, M., Lepage, L., Lucotte, M., Juneau, P., 2014. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. J. Exp. Bot. 65, 4691–4703. https://doi.org/10.1093/jxb/eru269.

Government of British Columbia, 2016. Brushing – Chemical Use (Air and Ground) graphic. Provided by the BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development, June 22, 2016.

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 inBC? Wildlife report no. 28. Ecosystems Branch, Ministry of Environment, Victoria, B.C. Available online: http://www.llbc.leg.bc.ca/public/pubdocs/bcdocs/442206/finishdownloaddocument.pdf (accessed 14 March 2019).

Health Canada, 2012. Consumer product safety: maximum residue limits for pesticides. Government of Canada, Ottawa, Ontario. Available from http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php (accessed 1 January 2021).

Kolakowski, B.M., Miller, L., Murray, A., Leclair, A., Bietlot, H., van de Riet, J.M., 2020. Analysis of Glyphosate Residues in Foods from the Canadian Retail Markets between 2015 and 2017. J. Agric. Food Chem. 2020 (68), 5201–5211.

Heap, I., Duke, S.O., 2018. Overview of glyphosate-resistant weeds worldwide: Overview of glyphosate-resistant weeds. Pest Manag. Sci. 74, 1040–1049. https://doi.org/10.1002/ps.4760.

Helander, M., Saloniemi, I., Saikkonen, K., 2012. Glyphosate in northern ecosystems. Trends Plant Sci. 17, 569–574. https://doi.org/10.1016/j.tplants.2012.05.008.

Henderson, A. M., Gervais, J. A., Luukinen, B., Buhl, K., Stone, D., Strid, A., Cross, A., Jenkins, J, 2010 (revised 2019). Glyphosate Technical Fact Sheet. National Pesticide Information Center, Oregon State University Extension Services. Available online: http://npic.orst.edu/factsheets/archive/glyphotech.html (accessed 28 December 2020).

Kissane, Z., Shephard, J.M., 2017. The rise of glyphosate and new opportunities for biosentinel early-warning studies: Rise of glyphosate. Conserv. Biol. 31, 1293–1300. https://doi.org/10.1111/cobi.12955.

Landrigan, P.J., Belpoggi, F., 2018. The need for independent research on the health effects of glyphosate-based herbicides. Environ. Health 17, 51–54. https://doi.org/10.1186/s12940-018-0392-z.

Larsson, M.O., Sloth Nielsen, V., Bjerre, N., Laporte, F., Cedergreen, N., 2018. Refined assessment and perspectives on the cumulative risk resulting from the dietary exposure to pesticide residues in the Danish population. Food Chem. Toxicol. 111, 207–267. https://doi.org/10.1016/j.fct.2017.11.020.

Lloyd, R., 1990. Herbicide effects on moose browse in northern British Columbia. FRDA Memo No. 161. BC Ministry of Forests, Smithers, BC. Available online: https://www.for.gov.bc.ca/hfd/pubs/Docs/Frm/Frm161.pdf (accessed 1 January 2021).

Machado, A.F.L., Ferreira, L.R., Santos, L.D.T., Santos, J.B., Ferreira, F.A., Viana, R.G., 2009. Absorption, Translocation and Radicular Glyphosate Exudation in *Eucalyptus* sp. Clones. Planta Daninha 27, 549–554. https://doi.org/10.1590/S0100-83582009000300016.

MacKinnon, A., Pojar, J., Coupe, R., Argus, G.W. (Eds.), 1999. Plants of northern British Columbia. Lone Pine Publishing, Edmonton, Alta.

Mamy, L., Barriuso, E., Gabrielle, B., 2016. Glyphosate fate in soils when arriving in plant residues. Chemosphere 154, 425–433. https://doi.org/10.1016/j.chemosphere.2016.03.104.

Meidinger, D.V., Pojar, J. (Eds.), 1991. Ecosystems of British Columbia, Special report series 6. Research Branch, Ministry of Forests, Victoria, B.C.

Newton, M., Horner, L.M., Cowell, J.E., White, D.E., Cole, E.C., 1994. Dissipation of Glyphosate and Aminomethylphosphonic Acid in North American Forests. J. Agric. Food Chem. 42, 1795–1802. https://doi.org/10.1021/jf00044a043.

Newton, M., Cole, E.C., Tinsley, I.J., 2008. Dissipation of four forest-use herbicides at high latitudes. Environ. Sci. Pollut. Res. 15, 573–583. https://doi.org/10.1007/s11356-008-0039-7.

Noyce, K.V., Garshelis, D.L., 2011. Seasonal migrations of black bears (*Ursus americanus*): causes and consequences. Behav. Ecol. Sociobiol. 65, 823–835. https://doi.org/10.1007/s00265-010-1086-x.

OECD, 1999. Consensus document on general information concerning the genes and their enzymes that confer tolerance to glyphosate herbicide. Series on harmonization of regulatory oversight in biotechnology. Report No. 10. OECD Publishing. https://doi.org/10.1787/9789264053465-14-en.

Oleskevich, C., Punja, Z.K., Shamoun, S.F., 1996. The biology of Canadian weeds. 105. *Rubus strigosus Michx., Rubus parviflorus Nutt.*, and *Rubus spectabilis Pursh*. Can. J. Plant Sci. 76, 187–201. https://doi.org/10.4141/cjps96-037.

Pinno, B.D., Landhäusser, S.M., Chow, P.S., Quideau, S.A., MacKenzie, M.D., 2013. Nutrient uptake and growth of fireweed (Chamerion angustifolium) on reclamation soils. Can. J. For. Res. 44, 1–7. https://doi.org/10.1139/cjfr-2013-0091.

Richmond, M.E., 2018. Glyphosate: A review of its global use, environmental impact, and potential health effects on humans and other species. J. Environ. Stud. Sci. 8, 416–434. https://doi.org/10.1007/s13412-018-0517-2.

Roy, D.N., Konar, S.K., Banerjee, S., Charles, D.A., Thompson, D.G., Prasad, R., 1989. Uptake and persistence of the herbicide glyphosate (Vision®) in fruit of wild blueberry and red raspberry. Can. J. For. Res. 19, 842–847. https://doi.org/10.1139/x89-128.

Stenrød, M., Eklo, O.M., Charnay, M.-P., Benoit, P., 2005. Effect of freezing and thawing on microbial activity and glyphosate degradation in two Norwegian soils. Pest Manag. Sci. 61, 887–898. https://doi.org/10.1002/ps.1107.

Székács, A., Darvas, B., 2012. Forty Years with Glyphosate. In: Hasaneen, M.N. (Ed.), Herbicides - Properties, Synthesis and Control of Weeds. InTech, Rijeka, Croatia, pp. 247–284. https://doi.org/10.5772/32491.

Thompson, D. G., Pitt, D. G., 2011. Frequently asked questions (FAQs) on the use of herbicides in Canadian forestry. Technical Note No. 112. Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, ON. Available online: https://cfs.nrcan.gc.ca/publications?id=32344 (accessed 1 January 2021).

Timms, K.P., Wood, L.J., 2020. Sub-lethal glyphosate disrupts photosynthetic efficiency and leaf morphology in fruit-producing plants, red raspberry (*Rubus idaeus*) and highbush cranberry (*Viburnum edule*). Global Ecol. Conserv. 24, e01319 https://doi.org/10.1016/j.gecco.2020.e01319.

Tong, M., Gao, W., Jiao, W., Zhou, J., Li, Y., He, L., Hou, R., 2017. Uptake, Translocation, Metabolism, and Distribution of Glyphosate in Nontarget Tea Plant (Camellia sinensis L.). J. Agric. Food Chem. 65, 7638–7646. https://doi.org/10.1021/acs.jafc.7b02474.

Viti, M.L., Alves, P.A.T., Mendes, K.F., Pimpinato, R.F., Guimarães, A.C.D., Tornisielo, V. L., 2019. Translocation and Root Exudation of Glyphosate by Urochloa brizantha and its Transport on Sugarcane and Citrus Seedlings. Planta Daninha 37. https://doi.org/10.1590/s0100-83582019370100030.

Wood, L.J., 2019. The presence of glyphosate in forest plants with different life strategies one year after application. Can. J. For. Res. 586–594 https://doi.org/10.1139/cjfr-2018-0331.

Wood, L., Botten, N., Werner, J., 2021. Glyphosate Residue Data, Northern British Columbia, 2018. Dryad, Dataset,. https://doi.org/10.5061/dryad.c2fqz617p.

Zach, R., Crichton, V.F.J., Stewart, J.M., Mayoh, K.R., 2011. Early winter food habits of Manitoba moose as determined by three rumen analysis methods. Can. J. Zool. https://doi.org/10.1139/z82-175.

Zhang, L., Rana, I., Shaffer, R.M., Taioli, E., Sheppard, L., 2019. Exposure to Glyphosate-Based Herbicides and Risk for Non-Hodgkin Lymphoma: A Meta-Analysis and Supporting Evidence. Mutation Res./Rev. Mutat. Res. https://doi.org/10.1016/j.mrrev.2019.02.001.

Zyoud, S., Waring, W., Al-Jabi, S., Sweileh, W., 2017. Global research production in glyphosate intoxication from 1978 to 2015: A bibliometric analysis. Hum. Exp. Toxicol. 36, 997–1006. https://doi.org/10.1177/0960327116678299.