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

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Abstract

Persistent non-lethal 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 (BC), 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 BC. 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 post-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.

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

1.0 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 hectares, with an average since year 2000 of approximately 17,000 ha/year (BC MoFLNRO, 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 percent of all aerially-applied herbicide applications and 57 percent of all ground-based spraying in the Province annually (Govindarajulu 2008). In forestry settings, aerial application of glyphosate may result in spray drift and incomplete application to plants in the understory. 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/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 (Bergstrom et al. 2011, Coupe et al. 2011). When glyphosate comes into contact with soil it binds to organic matter, iron and aluminum, and especially to clay particles (Miles & Moye, 1988). Adsorption of glyphosate to soil particles happens within the first hour after application (CCME, 2012). 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 & Moye, 1988)

(CCME, 2012). When bound to clay or metals in the soil matrix, glyphosate is somewhat stabilized (Al-Rajab & 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 Shepard 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 post-application (Ando et al. 2002). However, thorough research remains to be conducted across environmental gradients on the length of persistence of glyphosate post-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 biochemcial 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 : water partition coefficient (CCME 2012), indicating that it partitions into water and out of fatty acids and lipids (Harrison 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, Biocatalysts/Biodegradation Database: Glyphosate Pathway Map 2013), 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 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).

Non-target plants are, at times, subject to a treatment of glyphosate due to over-spray, spray drift, or simply because of their proximity to a targeted plant in a vegetation management scenario (Schrubbers 2016). When non-target plants are sprayed, it is often with low, non-toxic doses, because they did not receive a complete application. Research suggests that plants treated with non-toxic 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*), which has edible berries, and is a short, trailing plant to clasping twistedstalk (*Streptopus amplexifolius*), which also has edible berries and leaves, but grows between 0.5-1 m in height. Bunchberry may be in greater contact with soil or possess lower transportation requirements for ions to reach leaves compared to 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.

1.1 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, Figure 1), in order 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;

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, since no glyphosate was predicted to be in the plants sampled, and there is no known mechanism for the metabolic degradation of glyphosate in plants.

2.0 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 were similar in herbaceous and woody plants. Therefore, this study occurs over a three-year period of time and investigates glyphosate residue on different cut-blocks, to keep the time-since-application variable consistent.

Ten native plant species were targeted for sampling between 2013 and 2015, comprising 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).

168 2.1 Study Areas

169 Study areas were selected within the Peace River Region of BC according to local
170 herbicide application schedules. All sites targeted for sampling fell within the Boreal Black and
171 White Spruce moist warm (BWBSmw) biogeoclimatic zone. Sites were further described as
172 mesic-rich in nutrients and mesic to sub-hydric in moisture regime, and ranged in elevation
173 between 830-1100 m (DeLong et al. 2011). The soils in the areas sampled were classified as
174 brunisolic gray luvisols and lay above coarse, fragmented morainal till. Soil information was
175 obtained from maps and inventory surveys conducted in previous studies of the areas sampled
176 (Farstad et al. 1965, Lord et al. 1977, Alberta Research Council 1980, Smith et al. 2012,
177 Government of British Columbia 2017). In all areas sampled, soil texture and pH levels varied as
178 soil depths and horizons fluctuate (Table 1).

179 Study sites were treated with the glyphosate-based herbicide product VisionMax™, made
180 by Monsanto Canada Inc., one year prior to plant sampling in order to target and remove aspen
181 (*Populus tremuloides*) competition surrounding plantation conifers (Table 1, Figure 1). The
182 active ingredient in VisionMax™ liquid formulation is glyphosate, at a product concentration of
183 540 grams acid equivalent per litre, present as potassium salt. The formula is composed of 49%
184 potassium salt of glyphosate, 10% surfactant mixture, and 41% water. In VisionMax™, the
185 surfactant mixture is proprietary (Monsanto Canada 2011). Aerial applications were conducted
186 over the treated sites, with the exception of block 84-1, which was sprayed with a backpack
187 applicator over only a portion of the block. A prescribed four litres per hectare of VisionMax™
188 were sprayed once, uniformly over the treatment areas, at a concentration of 8% (4L chemical
189 formula added to 50 L water), following standard forestry operational procedures for herbicide
190 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 km/hr 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), where the average temperatures at the exact times of application ranged between 12.1 and 19.8 °C. Between 2012 and 2015 average August monthly temperatures for the region were 29.3 °C +/- 3.3 °C. 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 between 200-300 cm of snowpack and temperatures ranging from 2°C to -36°C between November and January (Environment Canada 2017), during which plants were dormant.

2.2 Plant Sampling

Six forest cut-blocks were targeted for plant collection one-year post-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 acicularuis* Lindl.), cow parsnip (*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-leaved 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, where only live leaves and/or fruit were sampled that would have been grown the season after application, or herbaceous perennials, where the entire shoot was newly grown 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 ID and treatment ID and frozen until prepared. After plant collection, sampled were brought back to Prince George, BC, where all plants were washed with water thoroughly to remove soil, and then oven-dried for 24-48 hours at 80^{o}C at the University of Northern British Columbia, 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 part 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 raspberry 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 University of Northern BC Analytical Chemistry Laboratory and to the University of Guelph Agriculture and Food Laboratory for analysis. Two labs were used for chemical analyses due to the unavailability of the University of Northern BC Analytical Chemistry Laboratory after the first year of study.

2.3 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 hours, and dichloromethane was used to clean 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.

2.4 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 was heavily clustered at zero due to the number of samples where 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 was conducted using non-parametric Mann-Whitney and Kruskal-Walis 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.

3.0 Results

No glyphosate or AMPA were detected in any of the control samples. No significant differences in glyphosate and AMPA levels detected were noted between treated sites ($p = 0.631$, $p = 0.281$ respectively), or between spray treatment dates ($p = 0.291$, $p = 0.139$, respectively) according to the Kruskal-Wallis test performed. Furthermore, when application date, collection date, and cutblock 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-square = 9.834, $p = 0.08$), indicating that only the intercept had a significant effect on the model.

Similarly, when modeling AMPA from treatment date and site number, results were insignificant (Likelihood ratio Chi-square = 7.315, p = 0.198). Therefore, the treated blocks x 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 variation 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 2012-2015, and this 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 evapo-transpiration mechanisms and general water movement through plants (Sharma and Singh 2001). Since a large amount of climatic variation is 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

306 different. Glyphosate was detected in shoots of 12 out of 23 plant samples across treatment
307 areas; where detected, average levels ranged from 76.5 to 1050 ppb. AMPA was detected in the
308 shoots of only 3 of 23 samples (Table 2); average levels detected ranged from 15.5 to 47.5 ppb
309 (Figure 2).

310 The roots of the plants analyzed showed significantly higher levels of glyphosate and
311 AMPA than the shoot structures sampled, across all species (Mann-Whitney test: $p < 0.001$ for
312 both glyphosate and AMPA by plant structure). Glyphosate was detected in roots of 20 out of 22
313 samples analyzed; concentrations ranging from 145 to 4350 ppb. AMPA was detected in 13 out
314 of 22 samples (Table 2). AMPA detected in roots ranged from 28 to 210 ppb (Figure 2).

315 Out of samples where glyphosate and AMPA were detected, herbaceous species with
316 shorter forms (< 50 cm height) were shown to have higher levels of glyphosate than woody
317 shrubs with taller forms (> 50 cm height) (Mann-Whitney U Test, $p = 0.053$) (Figure 2).
318 Herbaceous plants with taller forms were not significantly different in glyphosate content from
319 either shorter herbs or shrubs. No significant differences were noted in AMPA by height class.

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

326 Plants sampled were grouped by plant structure (fruit, shoot, or root) and by perennation
327 type (woody shrub - WS, non-woody evergreen perennial - NWE or herbaceous perennial - HP)
328 (Table 1). Statistically significant differences in glyphosate levels were detected between the

329 shoots and roots of woody and non-woody plants. The distribution of average glyphosate and
330 AMPA were different across categories of perennation and plant structure (Kruskal-Wallis test, p
331 < 0.001, $p = 0.005$ respectively) (Figure 3). According to the GLM, species and perennation
332 type-plant structure were deemed significant factors in glyphosate level detected (Likelihood
333 ratio Chi-square = 62.002, $p < 0.001$) whereas block was not significant (Wald Chi-square =
334 7.190, $p = 0.126$). According to GLM predicting AMPA level, only perennation type-plant
335 structure was deemed significant (Wald Chi-Square = 16.377, $p < 0.001$), whereas species and
336 block were insignificant.

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

344 ## 4.0 Discussion

345 ### 4.1 Glyphosate Detection

346 In order for glyphosate to be detected in perennial shoot tissue one-year after application
347 in northern environments, glyphosate molecules first must be stored in perennating tissues
348 through the dormant season, and then translocate to new shoots and leaves when growth is
349 initiated the following spring. The level of glyphosate detected in shoots was highest in
350 bunchberry, which was also the shortest plant sampled in terms of its above-ground height. Three
351 out of the four species where glyphosate was detected in shoot tissue, were less than 50 cm in

height from the soil (bunchberry, 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 is influencing the presence of glyphosate in shoot tissue in these species (Figure 2). Glyphosate was detected more frequently in root samples than shoot samples (Table 2, Figure 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). Since 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 & 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 high-bush cranberry, cow parsnip, 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 1990). Significant differences in persistent glyphosate and AMPA were shown between life strategies (Figure 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 like glyphosate compared to woody shrubs, because their perennation strategy involves the fundamental necessity to store all materials in the rootstalk for use the following year.

Two out of two blocks where berries were sampled tested positively for glyphosate. The berries collected were fresh and thereby uncontaminated by the treatment itself, and it follows that glyphosate was therefore translocated from other plant parts to the berries in the three cases found: twice in red raspberry, and once in oval-leaved blueberry. Levels detected were low; however, the presence of glyphosate in fruit a 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/harvesting fruit in areas where glyphosate has been applied in the past (Harrison 2007; Henderson et al. 2010).

Compared to 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 to 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 non-designated 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 non-toxic, 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 information is available about the possible presence of glyphosate in forest plant tissues.

4.2 Aminomethylphosphonic Acid Detection

AMPA was found in most root structures but only two shoot samples (Figure 2). The presence of AMPA may indicate the ability for glyphosate to be partially metabolized within tissue during the first year 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 (Kryuchova et al. 2014). In order 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. Clasping twistedstalk, red raspberry, cow parsnip, 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 non-woody plants (Table 2). These findings provide a basis for future genetic investigation of these species.

4.3 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 Tsaa Tse K'nai people (Bannister 2006) and the Gitksan Nation (Mackinnon et al. 1999). Cooked leaves of pink wintergreen were used by the Dene Tsaa Tse K'nai people as a wash to treat chickenpox (Bannister 2006). Young shoots of clasping twistedstalk 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 as a perfume (Mackinnon et al. 1999). The young stems of cow parsnip 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).

Communication that glyphosate will persist throughout the shoot and root systems of plants for at least one-year post-application should take place with 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).

444 Despite the operational best-practices used for the aerial application of glyphosate,
445 namely the control of spray drift and targeted dispersal via aircraft calibration, release height,
446 droplet size, and wind speeds, drift does still occur and off-target plant species both within and
447 outside targeted blocks are inevitably effected by herbicide treatments (Thompson et al. 2012).
448 The consistent detection of glyphosate in off-target plants (primarily growing underneath the
449 canopy of targeted aspen trees) demonstrated in this study illustrates the necessity of further
450 research into the duration of glyphosate persistence in plant tissues. Traditional-plant users
451 should take into consideration that the presence of glyphosate does not indicate toxicity,
452 however, more research is required on long-term, low-level persistent glyphosate before any
453 conclusion can be reached about its full direct and indirect impacts to ecosystem health.
454 Glyphosate applicators should increase awareness of glyphosate persistence so that plant users
455 can make an informed choice about their consumption. Currently within British Columbia,
456 signage is required to indicate that herbicide application has taken place on a given site. The
457 signage recommends no entry for a 24-hour period. It is recommended that the posted
458 information be re-evaluated to include longer-term information about glyphosate persistence in
459 plant tissues.

460 It may be possible to develop realistic guidelines or criteria for the use of glyphosate in
461 areas that have high value for berry-picking or plant use, allowing potential risks to plant
462 harvesters to be minimized. The development of a tool to estimate the likelihood of glyphosate
463 presence and translocation in plant tissue would help to guide managers in their use of
464 glyphosate, and would also provide information to public and First Nations about their exposure
465 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.

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Tables

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Table 1: Plant samples collected for glyphosate residue analysis in the Peace River Region of British Columbia along with location and date of collection. [1] References for soil information: Farstad et al. 1965, Lord et al. 1977, Alberta Research Council 1980, Smith et al. 2012, Government of British Columbia 2017.

Year sampled	Cut-block ID	Soil Series[1]	Clay % and pH range[1]	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 % clay and pH range 5.6-7.6	control	*Viburnum edule*	shrub	roots, leaves
					Rosa acicularis	shrub	roots, leaves
					Vaccinium ovalifolium	dwarf shrub	berries
					Rubus idaeous	shrub	berries
2013	84-1	Moberly Bisequa Gray Wooded loam and clay loams	10-30 % clay and pH range of 5.1 - 7.7	2012	*Viburnum edule*	shrub	roots, leaves
					Rosa acicularis	shrub	roots, leaves
					Rubus idaeous	shrub	roots, leaves
2014	188-1	Sundance Bisequa Gray Wooded loamy sand and sandy loam	10-20 % clay and a pH range of 5.2-7.6	2013	*Rosa acicularis*	shrub	roots, leaves
					Rubus idaeous	shrub	berries
2014	130-1	80 % Nose soil series sandy clay loam to sandy loams	5-20 % clay and pH range 5.6-7.6	2013	*Viburnum edule*	shrub	roots, leaves
					Rosa acicularis	shrub	roots, leaves
					Vaccinium ovalifolium	dwarf shrub	berries
					Rubus idaeous	shrub	berries
2015	635-5, 635-4, 635-4A	70% Pinto soil series and 30% Moberly soil series loamy sand - sandy loam	pH range of 4.7-7.0	2014	*Heracleum lanatum*	herbaceous perennial	roots, shoots
					Streptopus amplexifolius	herbaceous perennial	roots, shoots
					Cornus canadensis	evergreen perennial	roots, shoots
					Pyrola asarifolia	evergreen perennial	roots, shoots
					Petasites palmatus	herbaceous perennial	roots, shoots
					Galium triflorum	herbaceous perennial	roots, shoots

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*
Rosa acicularis	4/4	roots, shoots	0/4	NA
Viburnum edule	1/3	roots	0/3	NA
Vaccinium ovalifolium	1/1	berries	0/1	NA
Cornus canadensis	6/6	roots, shoots	4/6	roots, shoots
Streptopus amplexifolius	4/6	roots, shoots	3/6	roots
Heracleum lanatum	3/6	roots	2/6	roots
Galium triflorum	5/6	roots, shoots	3/6	roots, shoots
Rubus idaeous	2/2	berries	0/2	NA
Pyrola asarifolia	6/6	roots, shoots	1/6	roots
Petasites palmatus	2/6	roots	2/6	roots

*AMPA = aminomethylphosphonic acid

Figure Captions

Figure 1: Overview of locations used for plant sampling to detect potential glyphosate and AMPA residues post-application, in the Peace River Region of British Columbia.

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Figure 2: Glyphosate (circles) and aminomethylphosphonic acid (triangles) detected in root, shoot, and berry tissue 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 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.

Figure 3: Mean glyphosate levels detected in all samples, across all sample locations and years, grouped by plant structure and perennation type. WS = Woody Shrub, NWE = Non-Woody Evergreen, HP = Herbaceous Perennial.

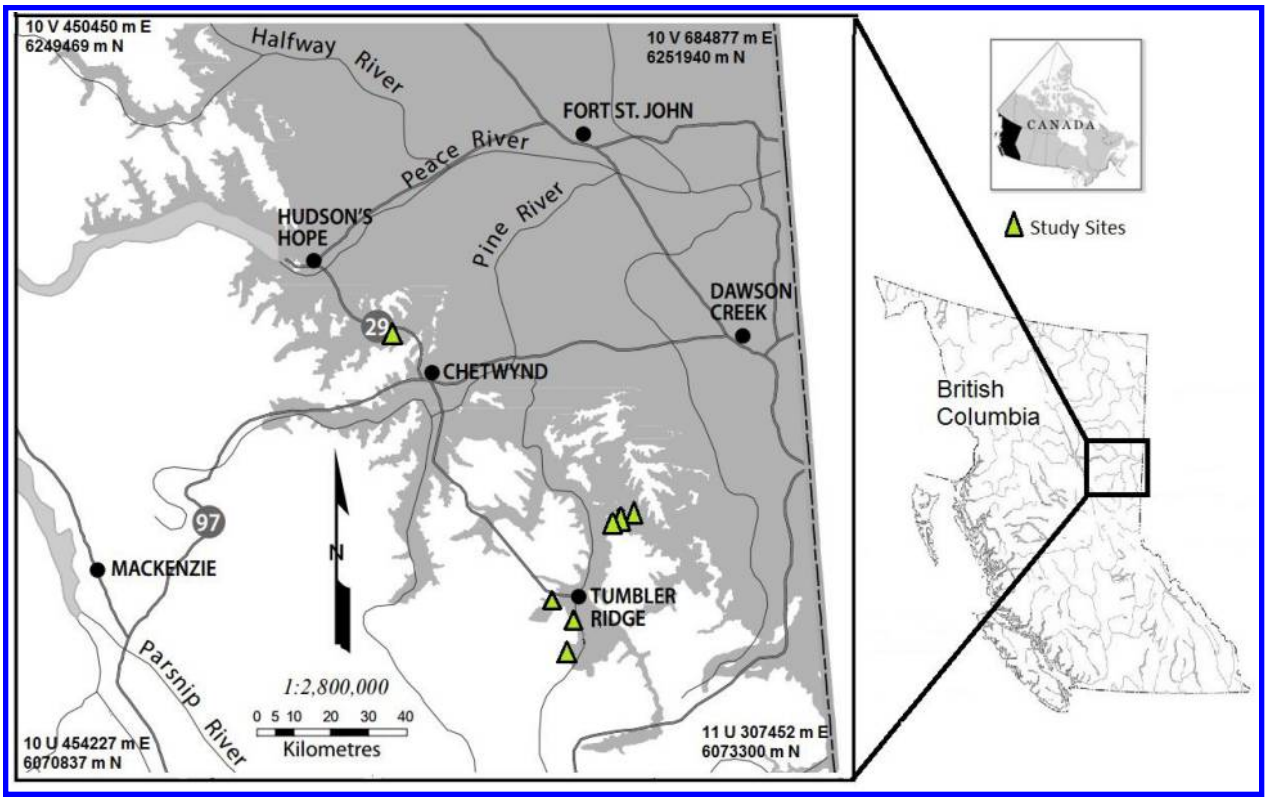

Figure 2

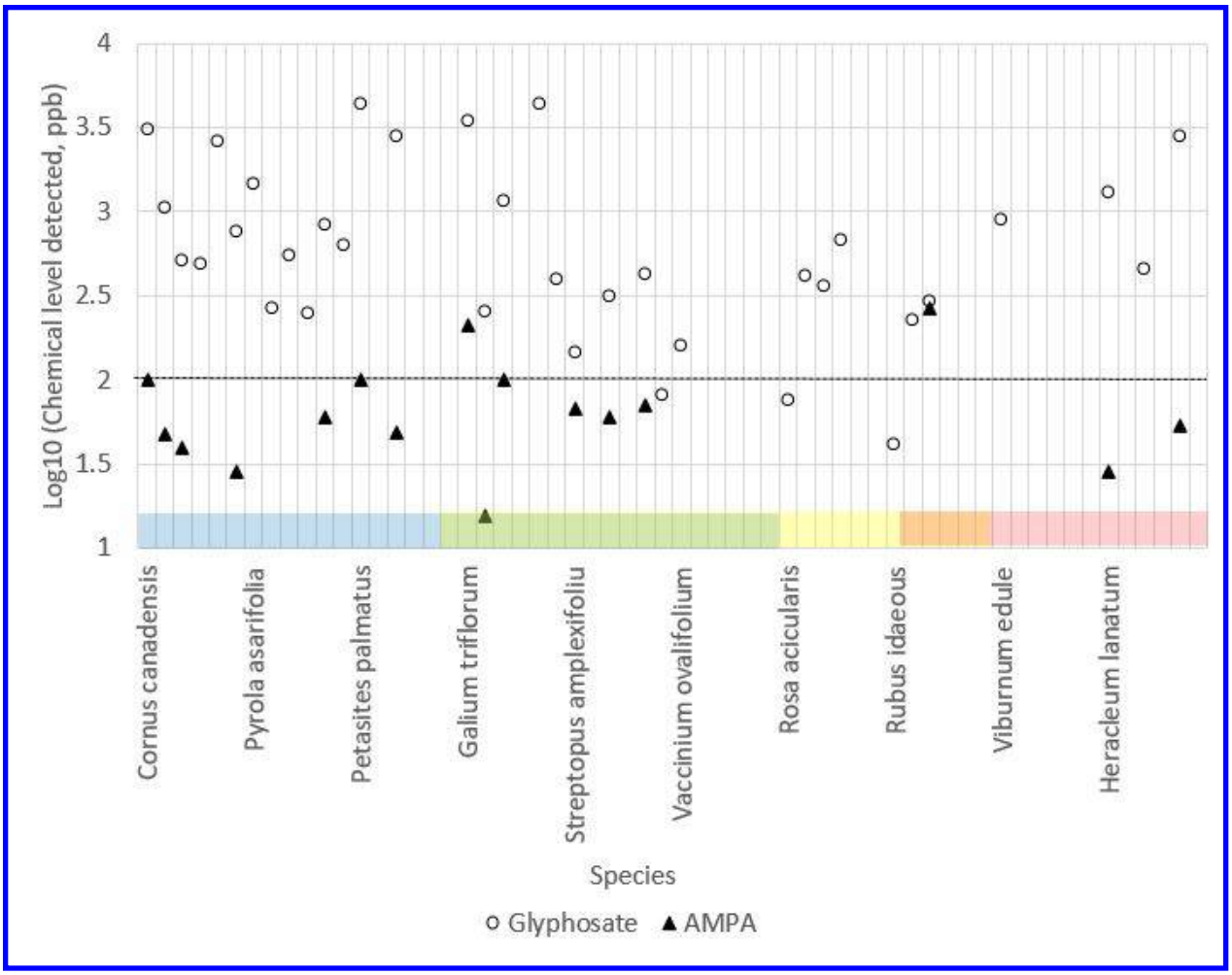

Figure 2

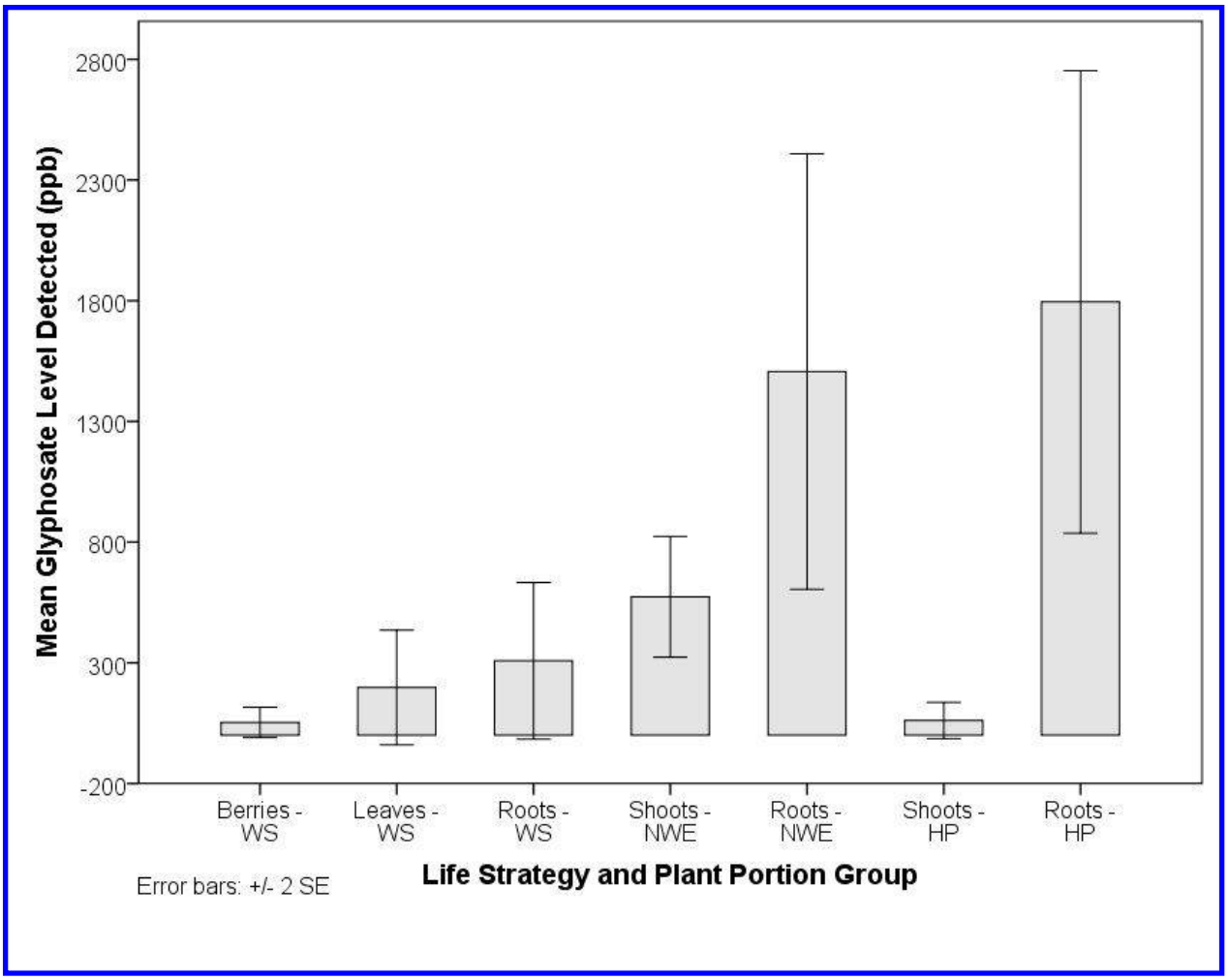

Figure 3