PLANT ALLOMETRY IN SPECIES PLANTED POST-GLYPHOSATE

APPLICATION, IN THE PEACE REGION OF BRITISH COLUMBIA

By

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ABSTRACT

The purpose of this study was to determine occurrence of glyphosate, and/or its primary metabolite AMPA, uptake and translocation within shrubby plant tissues. The study was conducted over the course of one year, and included monitoring plant response as well as testing for presence or absence of glyphosate in three plant species planted on a site post-glyphosate application compared to a control site. No traces of glyphosate or AMPA were present in the samples within the detection limit. The lack of glyphosate was attributed to the strong adsorption tendencies of glyphosate in soil types containing clay. There were, however, differences noted in various plant allometry when comparting plants on the treated site with the control after the one-year growing period. Differences observed in plant health and growth were explained by competition occurrence and exposure to weather, as well as potentially due to shifts in foraging behavior of wildlife on site. This study affirmed what was expected of the behavior of glyphosate in clay soils regarding adsorption; more research is required to support theories and questions about possible slower metabolic breakdown of glyphosate and the potential for glyphosate to accumulate in plants. This served as a preliminary study of glyphosate in northern British Columbian ecosystems.

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ABBREVIATIONS

AMPA	Aminomethylphosphonic acid
BC	British Columbia
C04	Control Plots (block 738-004)
G05	Treatment Plots (block 738-005)
HPLC MS/MS	High performance Liquid Chromatography and tandem Mass Spectrometry
PPB	Parts Per Billion
SEM	Standard Error of mean
SPE	Solid Phase Extraction

INTRODUCTION & BACKGROUND

Glyphosate is a non-selective herbicide used worldwide in agriculture and forestry (Al-Rajab and Hikami 2014). Herbicide is primarily used in forestry to control and kill broad leaf vegetation that is competitive to planted tree seedlings, and is also used as a site preparation tool prior to planting tree seedlings. Means of application include aerial spraying and individual tree application; in commercial forestry, both of these methods serve to eliminate potential vegetation competition. Aerial application of herbicide is the most common mode of controlling unwanted vegetation on an industrial scale because it is the most cost-effective way to achieve silvicultural goals (Fortier and Messier 2006). The practice of herbicide application for brush control is both criticized and widely researched. Generally, the public understands little about herbicide; the public assumes glyphosate unsafe, and its use is often met with opposition. Concerns have been raised about the possibility of potentially dangerous herbicide residuals remaining in the ecosystem and entering into plant tissues.

Glyphosate herbicide is a weak organic acid, water soluble and acutely toxic when administered to rodents (Health Canada 1987). The primary mode of decomposition for glyphosate is microbial degradation as it is resistant to chemical breakdown, relatively non-leachable, and stable in sunlight (Schuette 1998). The half-life of glyphosate is variable depending on conditions such as temperature and climate; soil studies conducted by the USDA found half-life ranging between 3 and 130 days (Schuette 1998). AMPA is the primary known degradation product of glyphosate (Simonsen et al. 2008).

Typically glyphosate is applied to vegetation to induce uptake through the leaves and from there, the chemical disrupts a key enzyme in the shikimic acid pathway used in plants, bacteria and fungi to produce essential aromatic amino acids (Glyphosate general fact sheet 2015). Although the mode of action for glyphosate is well understood (Shaner 2006), knowledge of dynamics of glyphosate activity and breakdown post-application are limited because of the site-specific nature of glyphosate's interaction with soil and plants. Glyphosate and AMPA in soil are assumed to be relatively immobile due to strong adsorption to soil particles (Schuette 1998), this limits availability of glyphosate for root uptake. In the absence of soil particles, Wagner et al. (2003) found that plants did have the capacity to absorb and uptake glyphosate when applied to roots of corn, and Sprankle et al (1975) found that wheat, corn and soybean could uptake glyphosate from sandy substrates in a laboratory setting. Glyphosate degradation and breakdown in a natural environment is dependent on soil microorganisms as well as site-specific factors (Schuette 1998), including temperature and soil moisture content. Johnson (2007) estimated that the chemical half-life of glyphosate increased by a factor of two or three with a 10°C decrease in temperature, as well as an approximate increase of the chemical half-life by a factor of one-half to two when the soil moisture content was reduced in half. Furthermore, once glyphosate has entered a plant via shoot or root tissue, the metabolization of glyphosate does not generally take place, due to the interruption of metabolic pathways (Duke et al. 2012).

Glyphosate is predominately applied aerially due to cost efficiency as well as the logistical advantage that aerial access provides (Thompson and Pitt 2003). Glyphosate is often applied in conjunction with other surfactants and adjuvants to assist in the adhesion and absorption into the plant (Setton and Palmer 2015).

There are many different stakeholders invested in British Columbian forests due to the perceived value of forests and the abundance of their distribution in the province. These values can be in stark contrast with each other and lead to conflicts in forest management and use (Burton et al. 2003). Public perception of safe forest management is relevant because in British Columbia 94% of the province is crown land (Ministry of Forests 2010), and of that, forests cover 62% (COFI 2016). Public perception of glyphosate use in forested areas of BC has historically been negative as the public generally understand very little about the dissipation, fate and allocation of glyphosate post-treatments. Concerns have been raised regarding the safety of using glyphosate in forests (FOEE 2013). Select provinces in Canada including Quebec and Nova Scotia have banned or stopped funding the commercial use of glyphosate (Robichaud and Phillips 2016). In 2015, glyphosate was added to the World Health Organizations list of probable carcinogens (Cressey 2015). This uncertainty is a growing concern as forests are becoming increasingly intensely managed for wood production; however, forest values include monetary value as well as experiential, ecological and cultural values. With greater management intensity and desire for faster tree rotation, an increased use in herbicides to control unwanted and competing vegetation is realized. All values must be taken into consideration in forest management; often the use of glyphosate as a management option does not synchronize with the values of other resource users, or the complexity of forest ecosystem dynamics.

It is commonly perceived that industrial use of herbicides for wood production will threaten the safety and sustainability of First Nations traditional land use practices and the ecological function of forests. First Nation practices include fishing, hunting, medicine collecting and berry picking; all of which are integral parts of First Nations culture

(Thompson and Pitt 2011). Since timber management areas and First Nations traditional lands are often in close proximity to one another or overlapping, the use of glyphosate for forest management, and presence post-application are of concern. Presence of glyphosate in areas that are extensively used and integral to First Nations could potentially lead to repeated exposure over extended periods of time. The purpose of this study was to address if glyphosate and its residual compounds are able to translocate into three different shrub species post-herbicide treatment, and therefore determine if the plant tissues become contaminated or remain useful for traditional sources of food and medicines.

The translocation and allocation of glyphosate within plants was expected to be variable. This is due to multiple site-dependent variables including: soil type, temperatures, plant species and moisture content. The study determined if any glyphosate within the detection limit was found in plant tissues after one year. Plant response and survival was also measured to compare allometry between sample species post-treatment. Investigations of the possibility of glyphosate uptake and translocation, as well as its effects on plant health and survival were conducted. This study determined the survival and growth of three planted species post-glyphosate application in order to determine how well these specific plant values can be maintained after the integration of glyphosate chemical into the soil environment. The purpose of this study was to determine if glyphosate [$C_3H_8NO_5P$] (N-phosphonomethyl) glycine) or aminomethylphosphonic acid [CH_6NO_3P] (AMPA) can be translocated from soil into plant tissue through root uptake.

METHODS

Study Area

This study took place on two sites approximately 15 km northwest of Chetwynd in the Peace region of British Columbia. Both sites were located in cut blocks where timber was harvested using conventional roadside logging methods and reforestation was required. The control site C04 (West Fraser Chetwynd cut block 738-004, 55° 47’ 02” N - 121° 48’ 18” W) was not treated with herbicide (Figure 1). The treatment site G05 (cut block 738-005, 55° 46’ 55” N -121° 49” 27’ W) was sprayed with glyphosate prior to planting (Figure 2). Both sites were located in the Sub-boreal Spruce wet-cool variant (SBSwk2) biogeoclimatic zone (BEC Program 2017). The mean annual temperature recorded at weather station ‘Chetwynd A’ is 2.9ºC ranging from -15.3ºC in January to 22.2 ºC in July with a mean annual precipitation of 418.9 mm (Environment Canada 2017). Soil survey maps (Agriculture Canada 1986, 1965) show soil are classified as Moberly series, Bisequa grey wooded loam and clay loam over morainal, cordillian till, in the area of the study.

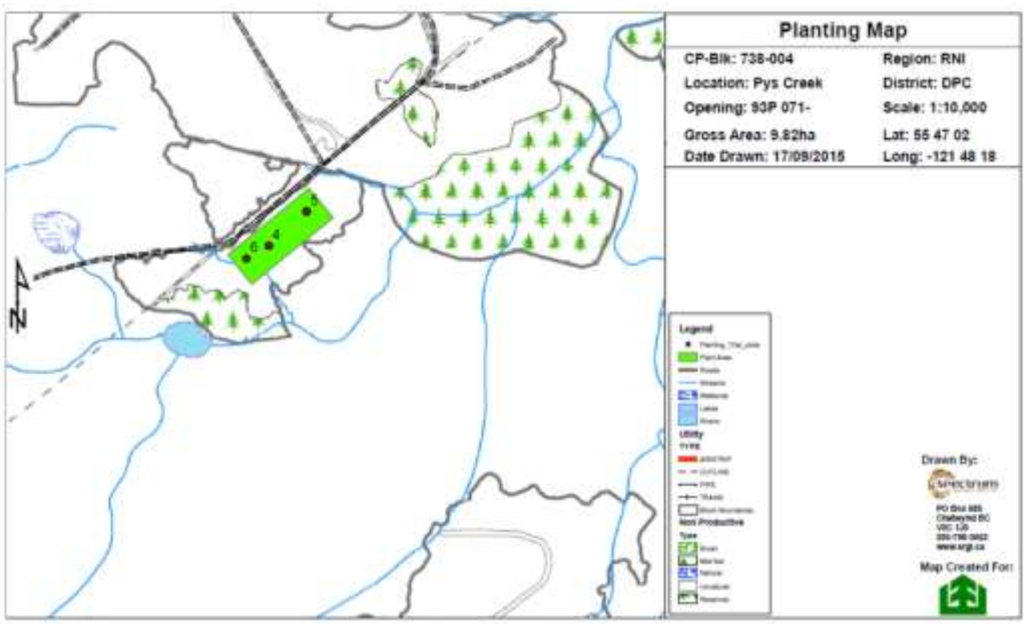

Figure 1. Planting map of control site and plot locations (4, 5 and 6) in cut block 738-004 (C04) northwest of Chetwynd BC.

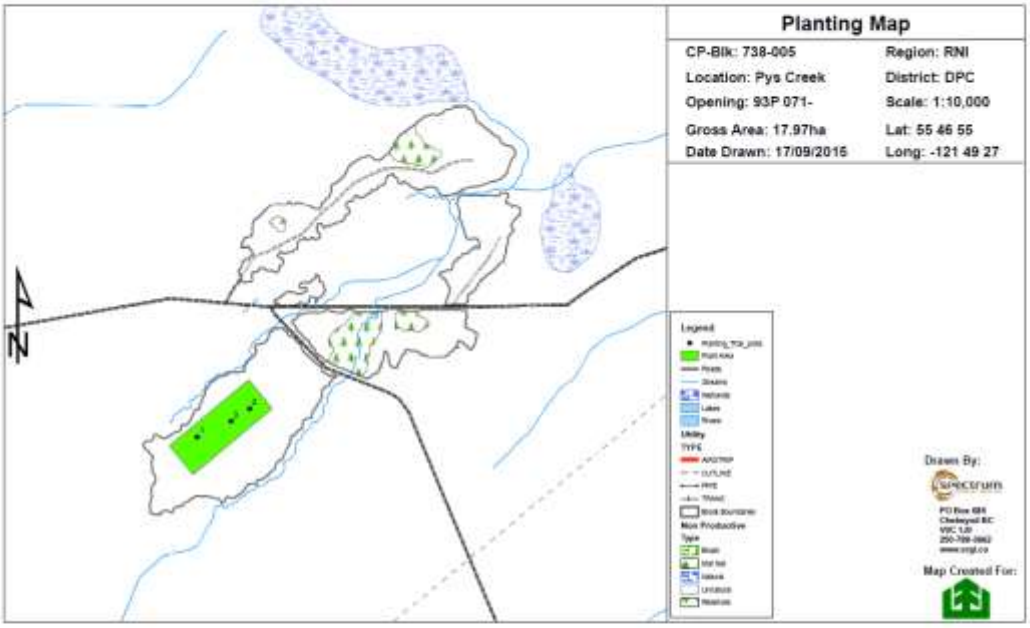

Figure 2. Planting map of treatment site and plot locations (1, 2 and 3) cut block 738-005 (G05) northwest of Chetwynd BC.

Experimental Design

Two 30m x 30m permanent plots were established, one in the glyphosate treated block G05, and one plot in C04. The control site C04 was not treated with herbicide, the treatment site G05 was aerially sprayed with a 4L/ha concentration of glyphosate herbicide formulation. After herbicide application, shrub species were planted on both G05 and C04. Three randomly-selected berry producing species were planted, they included: rattlesnake plantain (*Goodyera oblongifolia*), red raspberry (*Rubus idaeus*), and thimbleberry (*Rubus parviflorus*). Survey data were collected from these areas by measuring each plant within 3 x 3.99 m plots within the 30 m x 30 m permanent sampling area; plots 1, 2 and 3 (Figure 2) were in the treated area and plots 4, 5 and 6 were in the control area (Figure 1). Measurements were collected at the time of planting and one-year post-plant. Each plant was marked with a numbered pin in order to track changes in individual plants over time. Data collected included: species, height of plant, root collar diameter, competitive vegetation vigour, evidence of pathogens or animal browse, total unhealthy leaf area and an overall assessment code for the vigour of the targeted plant. These criteria were used in order to make comparisons of plant performance one year after herbicide application.

In order to determine glyphosate presence in the soil, four soil sample points were identified within the area to be treated and four within the control area for a total of eight soil samples. Sampling points within the area treatment were randomly selected and placed at least 10 m apart. The procedure for soil collection included removing the LFH layer, digging a small pit approximately 15 cm x 15 cm x 10 cm (L x W x H). The soil from the pit was placed in a large, clean bowls. The soil sample was randomly selected from the bowl by alternatively placing one scoop of soil in the sampling jar and discarding the next two scoops until the jar was full. Large debris such as rock, sticks and roots were removed as sampling occurred. All tools were thoroughly cleaned between samples, and sampling jars were sterilized prior to use by cleaning and rinsing with boiling water. Samples were labeled with the research plot number, sample number and date. Samples still containing some large debris were further sieved using a 2 mm mesh and returned to the sampling jar. Samples were frozen until sent for analysis.

A group of plants was sampled prior to planting, and analyzed using mass spectrometry to determine a baseline presence of glyphosate and/or AMPA. The other plants were then planted and left to grow for one year. After the one-year period a minimum of three samples of each species were collected to test for glyphosate and AMPA residue. Samples were frozen and taken to the Enhanced Forestry Lab Prince George, BC, for sample preparation. Preparation included dividing each plant into roots and shoots (foliage and stem), washing each sample thoroughly to ensure no contaminated soil was included in the sample, and then drying the samples at 80 degrees Celsius for 72-100 hours. Dried samples were ground into a fine powder using a mortar and pestle and blade grinder.

Prepared samples were sent to the University of Guelph Laboratory of Agriculture and Food to undergo chemical analysis. Vegetation samples were digested, then passed through a Solid Phase Extraction (SPE) cartridge to be analyzed using High performance Liquid Chromatography and tandem Mass Spectrometry (HPLC-MS/MS) to detect the presence of glyphosate or AMPA. The detection limit for normal testing using this method was approximately five parts per billion (ppb) for a five gram sample. Some of the plant samples collected weighed less than five grams; therefore, a detection limit for the data was estimated at approximately 25 ppb. Values determined through chemical analysis were compared to the initial baseline tests to determine any presence of glyphosate or AMPA in the plant tissue collected.

Plant performance was further defined based on the following variables: competition, vigour, growth rate and average height. All variables measured in treated plots were compared to those measured in control pots, measurements were also compared between years (2015 to 2016). The survival rate calculated was based on the presence or absence of plant samples after one year of growth. Mean heights after the growing period as well as mean growth rates for each species were calculated. Occurrence of competition, as well as occurrence of pathogens or browse were calculated by dividing the number of plant samples affected, by the total number of species present in the plot. After the one-year growing period, the data collection included assigning a code for the total leaf area dying as well as the overall vigour of each individual plant sample. Both of these codes were represented by a score of 0, 1, 2, 3 and 4 (Table 1).

Table 1. Plant criteria codes for data collection field crews when sampling sites G05 and C04 in years 2015 and 2016.

Does leaf size, seed production, colour, and overall productivity of competing vegetation indicate strong vigour?	Yes
	No
Is there evidence of pest/pathogens/animal browse on the vegetation being assessed?	Yes
	No
How much of the total leaf area is dead or dying? How much is chlorotic?	4) 0-25%
	3) 25-50%
	2) 50-75%
	1) 75-90%
	0) > 90%
Overall assessment code:	
Vigour excellent	4
Vigour good	3
Vigour fair	2
Vigour poor	1
Species dead	0

Data Analysis

The data analysis portion of this study can be divided into three categories: presence or absence of glyphosate, survival rates and plant allometry. Statistical analysis consisted of using a T-Test to determine whether the C04 plants were significantly different from the G05 plants with regard to the allometry measurements, competition and survival rates recorded. The null hypotheses stated that no significant difference would exist between two variables. If the t-statistic calculated was higher than the critical t-value at 95% confidence, then the null hypotheses were rejected, a difference between the two variables tested was concluded. Furthermore, a significance level of $\alpha = 0.05$ was used for all statistical testing, and if the p-value generated, was greater than 0.05, then the probability that the observed results were due simply to chance, was deemed too high for the result to be significant.

OBSERVATIONS AND RESULTS

Glyphosate Presence/Absence

No glyphosate or AMPA were detected in any of the plant samples after the one year growing period. Small concentrations of glyphosate and AMPA were detected initially in the baseline samples, which was unexpected; this finding will be addressed further in in the discussion section of this paper. Glyphosate was detected in the soil samples collected at concentrations of 0.05, 0.028, 0.042, 0.029 and 0.042 PPM in G04. Glyphosate and AMPA were not detected in C04 control area.

Plant Survival

The mean survival rate was calculated for each sample species based on presence or absence of plants after the growing period. The survival rates for plants in G05 were compared to plants in C04 (Figure 3). Confidence intervals of 95% were calculated for each species and are represented visually by error bars (Figure 3).

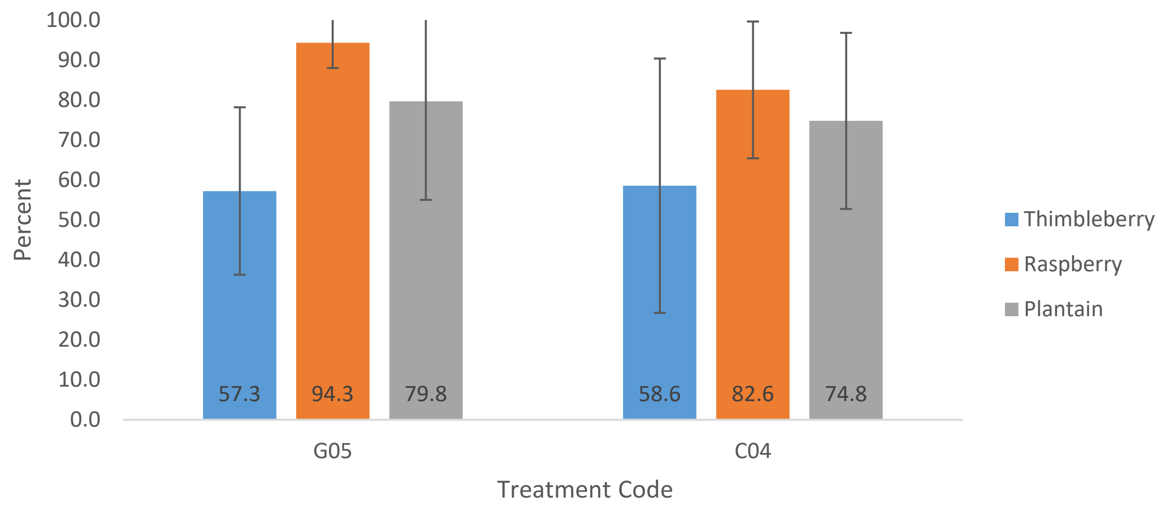

Figure 3. Mean survival rates of thimbleberry, raspberry and plantain in G05 (treated with glyphosate) compared with C04 (control) in 2016. One confidence interval of 95% displayed.

Although the survival rates in G05 showed slightly higher values compared with the survival rates in C04, the p-values resultant from the t-test were 0.92, 0.19 and 0.17 for thimbleberry, raspberry and plantain survival rates respectively. Of the reported p-values, none was below the < 0.05 threshold, therefore the t-test showed no statistically significant difference between plots sprayed with glyphosate and control plots with regard to survival rates.

<u>Plant Allometry</u>

Plant allometry measurements recorded in 2015 and 2016 were compared through statistical analysis and graphical representation. Nearly all plant growth variables collected showed no significant difference between G05 and C04. Of the seven categories of plant measurements, only competition occurrence reported a p-value lower < 0.05 suggesting significance, and computed a t-statistic where the null hypothesis of no significant difference, was rejected.

The mean height (cm) was calculated for each sample species based on the average heights of plants measured after the growing period. The heights were compared for both the G05 as well as the C04 area (Figure 4).

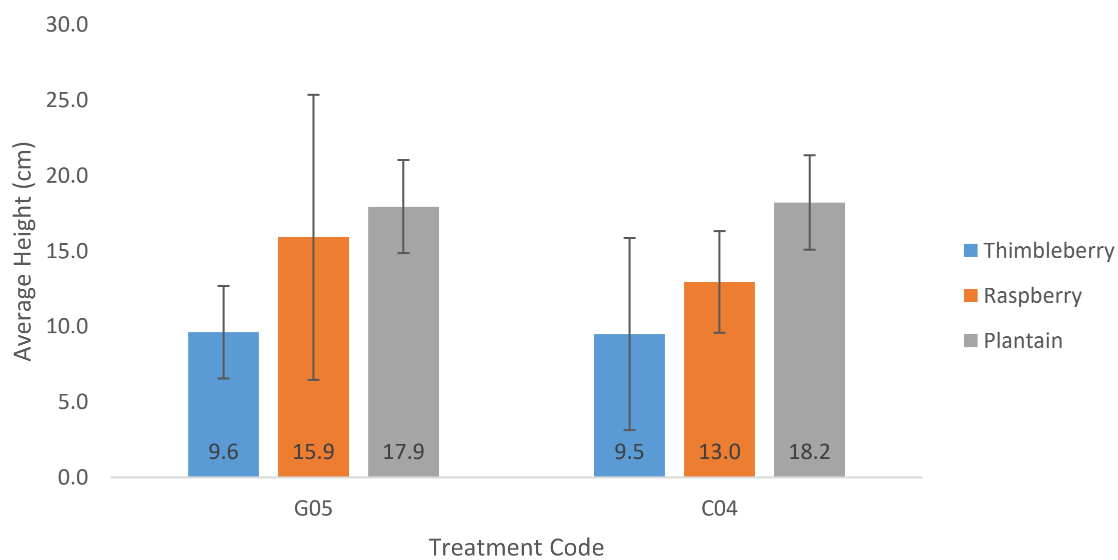

Figure 4. Average height of thimbleberry, raspberry and plantain in G05 (treated with glyphosate) compared with C04 (control) in 2016. One confidence interval of 95% displayed.

Although the mean heights in G05 had slightly higher values compared with the mean heights in C04, a t-test of the two data sets generated p-values that were insignificant for each species. P-values generated were 0.98, 0.60 and 0.77 for thimbleberry, raspberry and plantain respectively, therefore the t-test showed no statistical significant difference between G05 and C04 for mean plant heights.

Mean shoot growth for each species was calculated by measuring the difference in the height of the plant samples in years 2015 and 2016. Raspberry achieved the greatest mean growth rates in both the sprayed and control plots while thimbleberry had the lowest mean growth in both plots (Table 2). Plantain growth rates were in between the other two species growth rate values (Table 2).

Table 2. Average survival rate, height, and growth between thimbleberry, raspberry and plantain in G05 (treated with glyphosate) and C04 (control).

Block	Raspberry	Plantain	Thimbleberry
	Average Growth (cm/year)	**Average Growth (cm/year)**	**Average Growth (cm/year)**
G05 (Treatment)	6.44 ± 9.18	-1.42 ± 0.52	-6.10 ± 2.43
C04 (Control)	2.03± 2.43	-0.96 ± 3.26	-5.49 ± 5.39

Note: Values ±S.Dev. Negative growth values are possible in perennial plant species (plantain and thimbleberry) non-incremental growth.

A t-test was calculated for the growth rates of each species in G05 with C04 to determine any statistically significant differences. The p-values calculated for each species were 0.54, 0.88 and 0.06 for raspberry, thimbleberry and plantain respectively. Although plantain reported a p-value very close to being statistically significantly different at 0.06, of these none were below the threshold of < 0.05, therefore, there was no significance between G05 and C04 in terms of growth rate within species.

Competition occurrence around each plant was scored with either a code of Y (yes) meaning direct competition was present or N (no) competition was not present (Table 1). In all plots sampled there was 100% competition occurrence in the year 2015 shortly after samples were planted (Figure 5). After the one-year growing period, the competition occurrence was reduced in both G05 and C04. The competition occurrence was significantly lower in G05 versus C04. Confidence intervals of 95% for each species are represented by error bars in Figure 5.

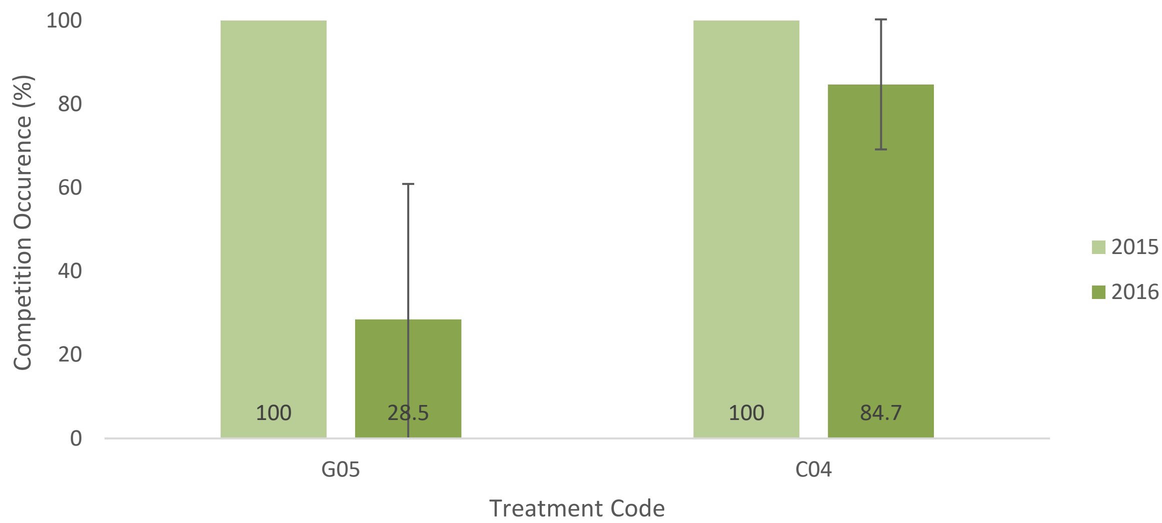

Figure 5. Percent occurrence of vegetation competition comparing G05 (treated with glyphosate) and C04 (control) blocks from years 2015 and 2016. One confidence interval of 95% displayed.

A t-test was performed for the competition occurrence in G05 versus C04 to determine if competition levels were significantly different between plots. The p-value calculated was 0.02, which is below the < 0.05 threshold, therefore the null hypothesis was rejected and there was a statistically significant difference between G05 and C04 regarding competition occurrence in 2016.

Occurrence of pathogen presence or browse was also noted and recorded with a code of Y (yes) meaning pathogen(s) or browse were present or N (no) meaning pathogen(s) or browse were not present (Table 1). The total number of Y codes assigned to plants was divided by the total number of plants in each treatment block. The year 2015 reported negligible levels of occurrence, whereas the year 2016 showed increased levels of occurrence with the greater amount occurring in G05 (Figure 6). Confidence intervals of 95% are represented by error bars in Figure 6.

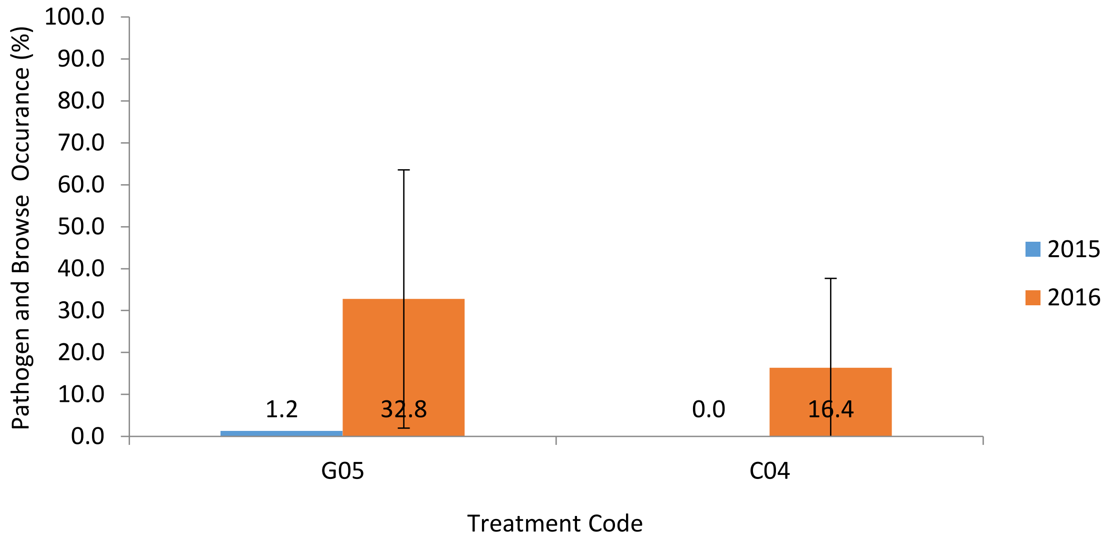

Figure 6. Occurrence of pest or pathogen presence codes in years 2015 and 2016 in G05 (treated with glyphosate) and C04 (control). One confidence interval of 95% displayed.

A t-test was performed for the pathogen and browse occurrence in G05 versus C04. The p-value calculated was 0.08, this value is close to statistically significant. However it is not below the threshold of < 0.05 therefore the null hypothesis was accepted and there was no statistical significant difference between G05 and C04 regarding pathogen or browse occurrence.

The amount of leaf area over the plant that was dying was assessed for each individual plant sample, and assigned a code ranging from 0-4. The codes represented differing leaf areas dead or dying, the codes include: 4 = 0-25%, 3 = 25-50%, 2 = 50-75%, 1 = 75-90% and 0 = > 90%. Firstly, the samples were separated into G05 and C04 (treatment and control) and then separated by species (Figure 7). Plants in G05 were generally distributed in greater percentages in the codes 3 & 4 where plants in C04 showed a more even distribution of the codes 0-4. Meaning more variety of healthy leaf area existed in C04.

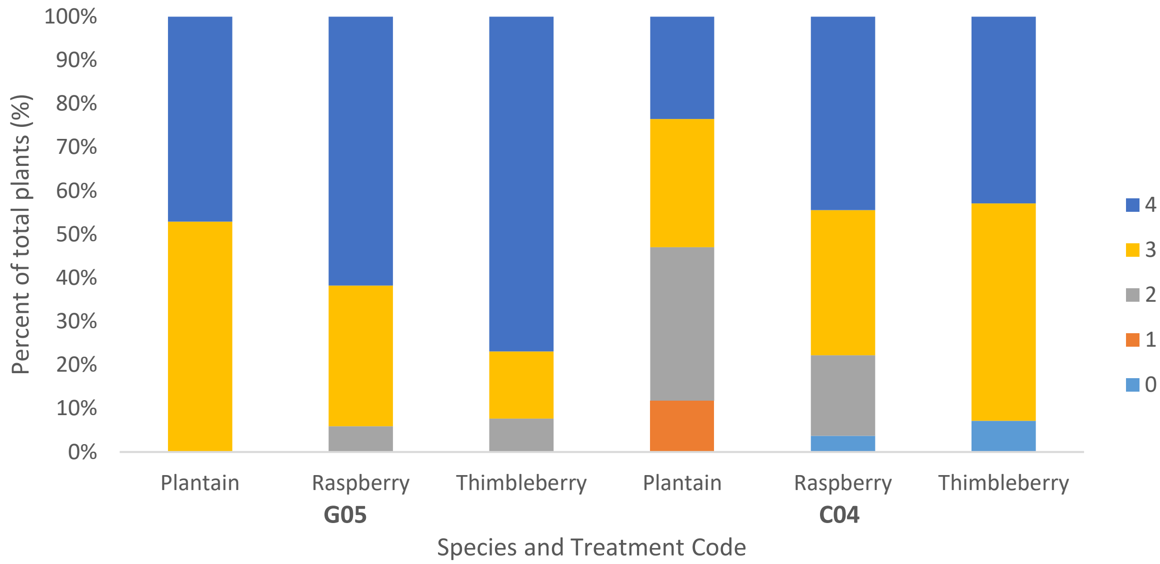

Figure 7. Leaf area dying scores by species compared in glyphosate G05 (treated with glyphosate) and C04 (control). Scores 4, 3, 2, 1, and 0 correspond to percentage leaf area dying (see table 1.) year 2016.

T-tests were performed comparing the species code distributions between G05 to C04, the p-values generated were 0.22, 0.42, 0.39, 0.83 and 0.04 for codes 0, 1, 2, 3, and 4, respectively. Of the p-values, one was below the threshold of < 0.05 and therefore the null hypothesis was rejected and there was statistical significant difference between G05 and C04 in terms of distribution of code ‘4’ measuring leaf area dying, G05 had a greater number of plants assigned a code of ‘4’ compared with C04. ANOVA testing was also conducted on the data set to further investigate significant difference between the leaf area dying code distributions. The distribution of codes in ANOVA demonstrated significant differences when comparing G05 and C04, the plants were generally scored as healthier (greater number of plants with a code of ‘4’) in the treatment compared with the control block.

Overall assessment codes were assigned to each species the codes ranged 4-0, each code represents different levels of vigour they include: 4 = vigour excellent, 3= vigour good, 2 = vigour fair, 1 = vigour poor and 0 = species dead. Firstly the samples were separated into G05 and C04 (treatment and control) and then separated by species (Figure 8). Plants in G05 were generally distributed in greater percentages in the codes 3 & 4 where plants in C04 showed generally a more even distribution of the codes 1-4. T-tests were performed comparing the species code distributions within G05 to C04, the p-values generated were 0.18, 0.74, 0.16 and 0.18 for codes 1, 2, 3, and 4 respectively. The code '0' was not compared using a t-test because no zeros were noted in the data collected. Of the p-values, none were below the threshold of < 0.05 and therefore the null hypothesis was accepted and there was no statistical difference between G05 and C04 in terms of distribution of codes measuring overall plant vigour.

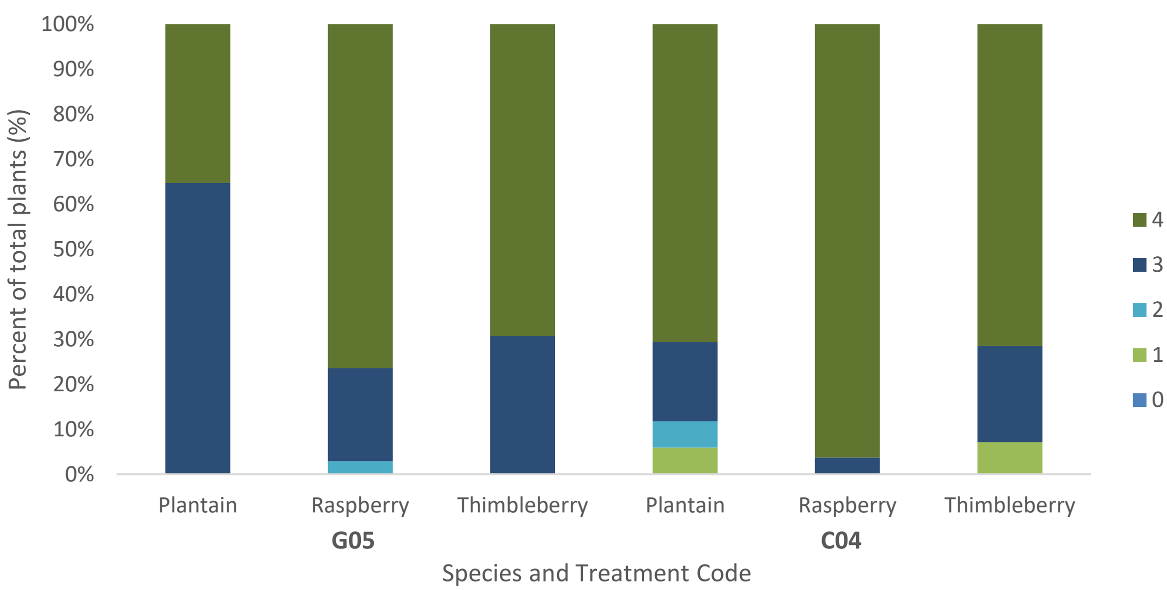

Figure 8. Overall vigour assessment scores by species compared in glyphosate G05 (treated with glyphosate) and C04 (control). Scores 4, 3, 2, 1, and 0 correspond to overall health of the plant (see Table 1.) year 2016.

DISCUSSION

Glyphosate Presence/Absence

After allowing plant samples to grow for one year in an area treated with glyphosate herbicide, it was expected that if plants had the ability to uptake and translocate herbicide, detectable levels would have been present in plant tissues. However, no glyphosate or AMPA were detected in the plant tissues after one year of growth. There are a range of explanations for these results.

One explanation is that the glyphosate could have killed any plants that translocated glyphosate and AMPA, and therefore, those plants were not measureable. Although this is a possibility, there were no significant differences in the survival rates between treatment and control plots, which indicated that mortality rates observed were unrelated to the treatment.

A second hypothesis is that the species investigated are unable to uptake glyphosate via root systems due to a biological control mechanism. Sprankle et al (1975) determined that soybean and corn did have the ability to uptake glyphosate through their roots in sandy soil beds; Wagner et al (2003) further supported this finding with evidence of glyphosate uptake by corn roots. Although it has been shown that glyphosate can be taken up by plant roots in some species, there is still the possibility that species-level differences exist in terms of root function and glyphosate translocation within plants.

Another theory, which explains the lack of glyphosate present in plant tissue samples after one growing period, relates to the strong adsorption tendencies of glyphosate in predominantly clay soil types (Glass 1987). Adsorption is the strong bonding of glyphosate molecules and AMPA to the soil particles thus making it essentially unavailable for root uptake and translocation in plants. Glyphosate molecular affinity for soil, and especially clay, would explain the lack of translocation into the plants sampled. The sites sampled for this study were classified as primarily calcareous loam (Moberly, Bisequa Grey Wooded loam and clay loam). This soil classification is generally considered to be composed of up to 24 inches of silt loam and loam (Farstad et al. 1965). Standard soil triangles report that silt loams and loams usually contain between 10% and 30% clay (Smith et al 2014). Furthermore, a main factor governing phosphorous adsorption is pH (Sidoli, Baran and Angulo-Jaramillo 2015), therefore not only should the classification of the soil be considered, but also its pH in determining if adsorption is likely to take place. The Moberly soil series profile is acidic in the upper 18 inches of soil, ranging from a pH of 5.6 to 5.9 (Farstad et al. 1965). Given that this 18 inches is where plant roots generally form, the combination of upwards of 30% clay content and an average pH of 5.75 will promote strong glyphosate adsorption to soil molecules, and thus yield it unavailable to plants for uptake via root systems in this area.

Glyphosate typically undergoes primarily microbial degradation in soil (Moshier and Penner 1978) and has been reported to breakdown over a period of 39-69 days in a study conducted in New Brunswick (Thompson et al 2000). Some recent work has highlighted the possibility that glyphosate in northern ecosystems does not break down as quickly as suggested in some laboratory and traditional glyphosate field studies (Helander, Saloniemi and Saikkonen 2012). Slower breakdown is due to slower metabolic processes of decomposition in colder temperatures and shorter growing seasons. This speculation needs to be further researched, to better understand how environmental factors are affecting the fate of glyphosate. In order to fully understand the potential for plants to uptake glyphosate via roots, its stability and fate within soil needs to be understood for cooler northern environments of varying soil types.

Unexpectedly, glyphosate residue was detected in the baseline plant samples analyzed at the University of Guelph Agriculture and Food Laboratory. The baseline samples were collected prior to ever being place in the ground, therefore the detection of glyphosate is somewhat unexplainable, and will have to be attributed to error. It is possible that some error exists in the HPLC MS/MS chemical analysis procedure at the chemistry laboratory, or that another phosphate molecule was detected that was interpreted as glyphosate. There is a very small possibility that pre-existing glyphosate was present in the plant samples from the nursery, however after a lengthy discussion with the facility management, this is very unlikely as no glyphosate is used in the nursery at all. One last possibility exists, that the native seeds collected by the nursery for propagation contained glyphosate from a previous industrial application in the collection area and glyphosate molecules were then passed on to the seedlings grown. It is unknown whether or not this process is scientifically possible, but there is evidence that transgenic glyphosate-resistance information can be passed from crops to wild plants in rice (Qiu 2013). Furthermore, Sprankle et al (1975) found that glyphosate presence did not interrupt seed germination in corn, soybean or wheat, indicating that seed germination may still be able to take place in contaminated environments, so the possibility of the transfer of glyphosate from seed to plant cannot be ignored.

Plant Survival and Allometry

Multiple factors influence plant growth at any one time, including light, water, mineral resources, and plant competition (Craine and Dybzinski 2013) all of which could apply to the plants measured in both the control plot as well as the treated area sampled in this study. No visual or statistical differences were noted in survival rates or overall plant height when comparing control to treated plots (Figures 3 and 4), indicating that the treatment had no effect on plant height or survival in the three species investigated in 2016.

Change in height of each species, or the growth, was more positive in the control plots for thimbleberry and plantain, and more positive for raspberry in the treated plots although growth was not statistically different between the control plot and treatment plot for any species. Results are insignificant and no trend can be distinguished for growth metrics (Table 2) further supporting the conclusion that the pre-planting glyphosate treatment had no effect on the three species studied.

Cedergreen (2008) found that non-toxic amounts of glyphosate administered to plants can actually stimulate growth. This phenomenon is known as hormesis, a biphasic response in plants or cells to varying concentrations of toxins. Mattson (2007) defined hormesis as “a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism”. However due to the lack of statistical significance in plant growth rate, height, and survival rates when compared between G05 and C04 (Table 2, Figure 3 and Figure 4) there is no evidence to suggest that and hormesis took place in this study.

The treated and control plots were significantly different in the level of competition occurrence (Figure 5). The difference could be attributable to glyphosate’s efficacy; it systematically kills broadleaf vegetation (Glyphosate general fact sheet 2015). Plant competition was eliminated in the treated plots allowing for more allocation of resources to the planted samples where as in the control plot, competitive vegetation hindered the growth and out-competed samples (Figure 5) (Craine and Dybzinski 2013).

Greater pathogen and browse occurrence took place on the planted species in the treated plots than those planted in the control, although the difference was statistically insignificant (Figure 6). Pathogen and browse occurrence was difficult to interpret due to the ambiguity of the category, combining the categories did not allow for definitive results to be drawn from the data, however the categorization provides an indication of the overall health of the planted species. Collecting more information regarding these characteristics is a potential area deserving future investigation. A study could attempt to see if animals show preferential browsing tendencies in areas sprayed with herbicide compared with areas left unsprayed. The increase in browse and pathogens in G05 compared with C04 (Figure 6) could possibly be due to a lack of preferred plant species in the treated plot on which the foragers would typically browse. However because this category measured all factors affecting health together instead of independently, it is impossible to draw conclusion regarding browse occurrence or foraging preference, and is one of the limitations in the study. Further study should be conducted on the pathogen occurrence as well as the browse occurrence on plants in areas both treated and untreated by herbicide applications. We can generally conclude from the health metric measured, as an amalgamation of agents such as pathogen, pest and browse damage, that the treatment lead to decreased health of targeted species in treated plots compared to control, however not a statistically significant decrease (Figure 6).

The percentage leaf area that was dead or dying showed that, in G05, leaf area was generally distributed greater in codes 3 and 4 (generally more leaf area alive), whereas C04 showed more variability within leaf area codes (Figure 7). Therefore we can conclude that there was more leaf area alive, as a total percentage of each plant measured, on targeted species in the treated plots than on targeted plants in the control plots (Figure 7). According to Figure 6 however, the leaf area that was alive in the treated plots was more likely to show pathogen, pest or browse damage. Furthermore, overall vigour assessment code also varied from G05 to C04 (Figure 8). In G05 the results showed no plants with codes of less than 2, indicating that all plants showed good (code 3) or excellent (code 4) vigour. Plants in C04 were assessed with codes ranging from 4-1, however there was a higher proportion of excellent vigour codes (4) in C04 compared to G05. Therefore, the overall vigour assessment shows that in C04, if a plant was heathy, it was doing really well (excellent), and in G05 if a plant was healthy it could have been struggling slightly but not enough to threaten future survival. Due to plant competition being one of the significant factors influencing sampled plant growth, the unhealthy plants with less vigour and greater proportion of leaf area dying could be potentially attributed to greater competition in the control site, C04. It also remains a strong possibility that, while the treated area plants were surviving, they showed decreased vigour compared to control plants due to increased browse or pest/pathogen pressure and/or due to increased exposure to climate elements.

RECOMMENDATIONS

Adsorption in soil means glyphosate competes with phosphate for binding sites on the soil or clay particle (Simonsen et al. 2008). Given that glyphosate and phosphorous sorb more readily to sites with higher clay content (Thompson 2012), areas of the British Columbia could be designated “safe” for glyphosate application. It would be helpful to study areas with predominantly different parent material to see if the same results take place in soils with less clay content where adsorption takes place differently. Research should also be done to determine how sorption of phosphates will take place according to pH classification around BC. A checklist of safety guidelines, or map of suitable locations for glyphosate use could be formulated based on the clay content and soil pH, thus linking these soil characteristics to a likelihood of glyphosate translocation, or a risk factor for glyphosate movement through ecosystem components such as water, plant tissue, or animal tissue. This information could potentially help managers more clearly understand the disruption glyphosate may have on an ecosystem. The study sites examined in this research may fall under the "safe for use" category, given the clay content and pH level of the Moberly soil series and the coordinated findings presented here which indicated no treatment impact to the shrubs planted post-glyphosate application.

However, caution should remain when administering chemicals to the forest in order to achieve short-term goals. The study presented illustrates a preliminary investigation of plant-soil-glyphosate interactions in northern environments. The implications and possibilities of accumulation of glyphosate and unintended effects are still not fully understood, and should leave silviculturists wary. Although glyphosate can be used as an effective and powerful tool, its use may compromise other forest values. Concern about the wide use of glyphosate across BC is warranted. Though herbicide is a powerful and effective tool, it is not appropriate in every situation and the designation of "safe" needs to be fully researched in a greater capacity than what is presented here prior to implementation. It should be considered that it is possible for glyphosate to desorb once it is tightly adsorbed to soil (Mamy and Barriuso 2007). Glyphosate is seldom applied on its own, it needs surfactants too ensure it sticks to the leaves as well as adjuvants, although these are considered inert because they do not affect plants, they are often toxic to humans (Mesnage et al 2014).

A multitude of questions still remain to be answered after this research was conducted, they include investigating how the environmental fate of glyphosate is affected by northern soils and ecosystems. Other environmental factors could have contributed significantly to the results of the study. Precipitation increased from 418 mm average to 564.1 mm in 2016 which is a 25.8% difference (The Weather Network 2017). The implications and effects this increase in precipitation are difficult to quantify. Higher amounts of precipitation can lead to greater run off, or in some microsites, pooling of stagnant water. In order to attempt to get more accurate results regardless of random weather events, the same study could be conducted multiple years successively. Another interesting avenue of exploration concerns the interaction of glyphosate with other soil minerals.

Future study could examine shorter time periods. Due to the variable half-life of glyphosate and its assumed fast microbial breakdown rate (Schuette 1998), investigations could take place on varying time scales including trials that last hours, or multiple days in an attempt to capture plant response over short time periods.

Also, a study which attempted to eliminate competition from the equation because of its significance would help to narrow down the impacts of the glyphosate chemical itself. For example, mechanically removing competitive plant species from the control area to mimic plant density in the treated area. However, this would not allow for the study of how a change in plant composition impacts foraging behavior or pest/pathogen attack in treated areas.

CONCLUSION

Glyphosate was not able to translocate into plant tissues studied, through root uptake. Glyphosate was most likely adsorbed tightly to the soil at our study sites and was not detected in the samples in this study. Therefore we determined that the plant tissues at the study sites presented here (raspberry, thimbleberry, and plantain from GO5 and C04) were not contaminated and remain useful for traditional sources of food and medicines.

It remains possible that the plant species selected in this experiment have the capability to uptake glyphosate, depending on the soil substrate in which they are growing. Effects of glyphosate treatment were manifested in the elimination of competitive vegetation on sites which lead to slightly healthier plants in the treatment compared with the control, as well as less variety in the distribution of plant health codes. However, differences in survival rates, growth, height, and pathogen/browse occurrence all showed no significant difference.

In clay dominated sites, such as the ones investigated in this study, the application of glyphosate could be appropriate if desired results are elimination of broad leaf vegetation. This result assumes that the high-percentage clay content of the soils in our study area caused glyphosate to adsorb so tightly to the soil matrix, that it became unavailable for plant uptake. Since no investigation was conducted on soil translocation of glyphosate, or on the potential dangers to other soil life, the conclusion that glyphosate use may be acceptable in areas with clay soils is limited to its effects on native plants.

The lack of glyphosate in the samples of this study does not mean that, on other sites, the translocation of glyphosate may not take place. It is reasonable to expect that in other soil types, with less clay, that glyphosate will be freer to move through soil and/or into plant tissues via roots. More research, such as conducted in this study, should be pursued specifically in northern soils where glyphosate degradation rates and processes are different than those typically associated with glyphosate safety testing.

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