EARLY RESPONSES OF NORTHERN BOREAL VEGETATION TO POWER LINE RIGHT-OF-WAY MANAGEMENT TECHNIQUES INCLUDING THE ACUTE TOXICITY OF IMAZAPYR AND TRICLOPYR TO NON-TARGET PLANTS

A Thesis Submitted to the College of Graduate Studies and Research In Partial Fulfillment of the Requirements For the Degree of Master of Science In the Department of Plant Sciences University of Saskatchewan Saskatoon

By

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Abstract

Integrated Vegetation Management (IVM) techniques on power line rights-of-way (ROWs) have successfully reduced environmental and economic costs of vegetation management by selecting techniques that facilitate the establishment of stable, low-growing plant communities. To test whether IVM principles can be applied to ROWs in northern Canada, I investigated the impacts of eight management strategies on plant communities in Yukon, Canada. Because forestry herbicide applications are not common in Yukon, I also examined the acute toxicity of imazapyr and triclopyr to non-target plants in standardized greenhouse tests with common boreal herbs. For treatments, triclopyr and imazapyr were each applied by three methods: broadcast spray, cut stump and point injection. Additional treatments were mechanical mowing or cutting target species and seeding native grasses. Vegetation cover surveys were completed before treatments and repeated after one year along with visual herbicide damage assessments. ROW plant communities were significantly altered by management methods one year after treatment, but clear directional changes were not yet evident. Herbicide treatments were more effective at target species control than mechanical methods. All treatments caused a minor reduction in nontarget species cover. Imazapyr applications caused more damage to non-target species than triclopyr. Other treatment impacts were life form (e.g. shrub, forb, etc.) or species-specific. Vegetative vigour tests and seedling emergence and seedling growth tests in five ROW soils were used to assess toxicity of both herbicides to Achillea millefolium and Chamerion angustifolium. Test results supported field findings: imazapyr was more toxic than triclopyr. Foliar Inhibition Concentration (IC)₅₀ estimates were 0.7 and 1.2% of the maximum imazapyr application rate vs. 31% for A. millefolium and triclopyr (C. angustifolium's could not be calculated). Soil applied triclopyr caused IC₅₀ estimates of 2-20 μ g g⁻¹ and imazapyr IC₅₀ estimates were $<2 \mu g g^{-1}$. Generally, each species was similarly sensitive to each herbicide and each herbicide was similarly toxic in each soil. A. millefolium performed well as a test organism in both tests. The differences in life form/species responses to treatments strongly suggest that shifts in plant community development have been initiated. Imazapyr's high phytotoxicity and persistence in soil indicates the herbicide is not a suitable product for northern ROWs if maintaining non-target vegetation is a management priority. An additional study on triclopyr dissipation in plant tissue found >50% of residues remained after 30 days and indicates further research into triclopyr dissipation and risks to wildlife in northern ecosystems is needed.

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1.0 INTRODUCTION

Electrical utility rights-of-way (ROWs) present unique and demanding challenges for vegetation management. Utility companies are required to provide safe, reliable service which can be compromised by trees near or underneath the transmission lines. Adjacent forests provide locally adapted seeds and suckers to rapidly recolonize the ROW which results in a cyclical management regime of tree/tall shrub removal (Berkowitz et al. 1995). Prior to the 1940s, mechanical methods of brushing, mowing, or hand cutting were the only tools utilized. After the Second World War, herbicide use became more common and is now widely used in North America (Sulak and Kielbaso 2000). Promoting interspecific competition through seeding or enhancing shrubby or grass species is a relatively new technique that may also be used to supplement either mechanical or chemical treatments (Meilleur et al. 1997). With this increased toolbox, ROW managers have much more complex treatment options to evaluate and implement. The term "Integrated Vegetation Management" (IVM) is applied to this decision matrix and implies that no one treatment is going to be effective for all sites and situations and many factors must be taken into consideration. The first step in developing an IVM plan is to establish a thorough understanding of local plant community dynamics and how they are affected by different management methods (Nowak and Ballard 2005).

There are more than 1000 km of power line right-of-way (ROW) in Yukon, Canada. Vegetation management along the 30 m wide corridors has historically been by mechanical methods using heavy equipment to mow or brush the vegetation. As part of a larger research project investigating potential IVM strategies for Yukon ROWs, the objective of this thesis is to examine management impacts on target and non-target vascular plants one year after treatment. Specifically, this involves documenting how mechanical, chemical and biological management techniques influence plant communities, the short-term efficacy of treatments on target species control and the response of non-target species in terms of cover change and herbicide induced damage. Herbicide use is not common in Yukon and a detailed assessment of herbicide phytotoxicity to two common herbaceous species using standardized acute toxicity testing methods is also included.

Chapter 2 reviews the principles of designing disturbance to meet vegetation management objectives, current vegetation management options for power line ROWs, and potential IVM strategies for Yukon ROWs. Knowledge of herbicide behaviour in the North is minimal and

there are additional management considerations beyond efficacy of herbicides on target species; the impacts of herbicide applications on non-target species are of particular concern. Limited information on the phytotoxicity of imazapyr and triclopyr to boreal species is available and the use of acute toxicity tests to provide detailed phytotoxicity information is discussed. Current testing methods and limitations are also explored. In addition to herbicide phytotoxicity to non-target plants, the risks of herbicide in plant tissues are also highlighted. Chapter 3 investigates the changes to vascular plant species composition and abundance one year after eight ROW management treatments. The chapter demonstrates that chemical methods are more effective at short-term control of woody target species than mechanical or biological manipulations. Herbicide treatments, however, also had significant, and in some cases persistent, adverse effects on non-target plants. Chapter 4 examines the sensitivity of the two most abundant and frequent forbs at the field sites to the two herbicides tested in the field. Fifty percent inhibition concentrations (IC₅₀) were well below field application rates, with significant differences between species and between herbicides. Chapter 5 provides a summary of the main findings, discusses management implications and outlines topics that would benefit from further research.

1.1 References

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2.0 LITERATURE REVIEW

The objective of this literature review is to examine the fundamental vegetation management principles behind integrated vegetation management (IVM) and the current management options for power line ROWs. How these principles can be applied and adapted for vegetation management on Yukon ROWs is also investigated. Understanding how plant species are impacted by herbicide applications is a critical component of IVM. As a tool for determining individual plant species sensitivities to herbicides, the application and limitations of dose-response relationships and phytotoxicity testing is discussed. In addition to direct impacts of herbicide applications on plants and plant communities, persistence of herbicide active ingredients in plant tissue can provide a vector into other ecosystem compartments. The potential for herbicide persistence in vegetation in northern conditions is briefly discussed.

2.1 Designing Disturbance to Meet Vegetation Management Objectives

2.1.1 Response of vascular plant species to disturbance

The process of manipulating disturbance type, size and intensity to promote the establishment of desired plant species or communities was first described as "designing" disturbance by Rosenberg and Freedman (1984). To design disturbance and thus promote plant communities to meet management objectives, the first task is to understand the local ecosystem and how plant species respond to disturbance (Pickett et al. 2009).

There are a number of species' attributes that influence post-disturbance community composition (Noble and Slatyer 1980). Firstly, the method of species arrival and persistence both during and after disturbance determines the availability of potential colonizers. Many boreal species rely primarily on clonal growth strategies and are therefore significantly impacted by the size of disturbance more acutely than to species relying on wind for seed dispersal if belowground systems are destroyed (Rydgren et al. 2004). ROWs in Yukon are generally 30 m wide, which is well within the clonal dispersal range of woody species such as *Populus tremuloides* whose clones can spread over multiple hectares (Kemperman and Barnes 1976). The type and intensity of disturbance can also influence the persistence of species. For example, in a clearcut with only aboveground disturbance, *P. tremuloides* can regenerate in very high densities from only a few individuals as the type of disturbance did not overly interfere with regeneration by suckering (Ilisson and Chen 2009). In contrast, Peltzer and others (2000) found that increasing soil disturbance intensity significantly reduced the shoot mass and stem density of *P*.

tremuloides by reducing the viability of suckers. Disturbance intensity can also alter species composition in the boreal seedbank (Lee 2004). Previous land use has also been identified as a major factor as industrial or agricultural activities impact the composition of the seed bank and availability of propagules at a site (Rydgren et al. 2004). This may be less important on northern ROWs as there are few, if any, areas that had prior intensive land use.

Once species have arrived on site, their persistence depends on a second set of attributes that determines the species' abilities to establish and grow to maturity within the ecological conditions of the early community (Noble and Slatyer 1980). There are two common strategies used by plants: they either rapidly uptake available resources or persist and grow with limited resources (Ballandier et al. 2006). Following a disturbance event there is typically an abundance of resources and this favours species with aggressive resource use strategies (Noble and Slatyer 1980) such as *Chamerion angustifolium* and *Calamagrostis canadensis* (Hangs et al. 2003). The majority of northern pioneer species are shade intolerant and competition for light is critical in early successional communities (Kembel and Dale 2006; Man et al. 2008). Soil nitrogen is also a common growth-limiting resource in boreal ecosystems and below-ground competition plays an important role in community development (Man et al. 2008). Interactions between species as they recover from disturbance may also transition between competition and facilitation as conditions change (Holmgren et al. 1997). For example, increasing herbaceous cover reduces light availability, but may improve moisture retention and create a more favourable seed bed for some species (Holmgren et al. 1997). The production and excretion of allelochemicals can also influence species performance after establishment. In the boreal forest, the dwarf shrub Empetrum nigrum spp. hermaphroditum has demonstrated allelopathic inhibition of Pinus silvestris and Populus tremula germination (Zackrisson and Nilsson 1992). Reduced Picea mariana establishment and growth near Kalmia angustifolia plants also suggests potential interference through a belowground mechanism (Inderjit and Mallik 2002). Beyond plant community interactions, stressful environmental conditions, herbivores and pests can also influence species persistence (Pickett et al. 2009). Finally, the length of time between different life stages impacts the continuance of a species at a given location. This includes time required to reach a reproductive state, the entire lifespan of a species and the longevity of propagules in the environment (Noble and Slatyer 1980).

There are many factors involved in plant community development, but disturbance history consistently has a major influence (Attiwill 1994; Rydgren et al. 2004; Schmitz et al. 2006). In the northern boreal forest, this has been demonstrated by differences in communities after varying fire regimes or harvesting practices (Johnstone 2006; Macdonald and Fenniak 2007). As discussed above, each plant species will react to disturbance differently. By altering the type, frequency and intensity of disturbance, plant communities can effectively be designed to meet management objectives (Attiwill 1994; Pickett et al. 2009). Integrated Vegetation Management (IVM) was founded on these principles; the history and development of IVM is discussed in the next section.

2.1.2 The history of integrated vegetation management for ROWs

When Egler (1954) first proposed the concept of Initial Floristic Composition, the theory provided an explanation for the existence of multiple plant community equilibria within a relatively homogenous site rather than a single stable climax. The principle of Initial Floristic Composition states that after disturbance, plant species of all successional stages already exist on site as propagules and come into dominance through a series of developmental stages (Egler 1954). If a group of species, such as trees, are essentially removed by management actions like herbicide use, the plant community is fundamentally altered and can transition into new, potentially stable states (Egler 1954). Initial Floristic Composition facilitated a vegetation management paradigm shift from simply "resetting" succession to intentionally modifying community development to achieve an alternative, desirable stable state (Rosenberg and Freedman 1984; Niering 1987). It was during this time that Egler and other researchers studying rights-of-way management (ROWs) began to recognize and document changes in plant communities after different ROW treatments (Niering 1958). Both Bramble and Byrnes (1983) and Niering and Goodwin (1974) reported low growing shrub communities as both stable and desirable covers for ROWs, linking high shrub density with reduced tree invasion. The mechanisms of how ecosystems resisted the regrowth or invasion of target species were not always clear, but the success of shrub covers were consistently related to high stem densities and canopy cover of erect shrubs. Further studies into the relationship between shrub cover and reduced tree growth identified selective herbicide application as the most effective method of achieving desirable shrub communities (Dreyer and Niering 1986; Niering et al. 1986; Bramble et al. 1991; Mercier et al. 2001; Yahner and Hutnik 2004). As knowledge of the dynamics

between plant communities and management methods increased, best practices for ROW vegetation management evolved from mowing, to indiscriminate herbicide applications, and eventually into an effective pest management strategy: Integrated Vegetation Management (IVM) (McLoughlin 2014). IVM is now a sophisticated integrated pest management system of implementation, monitoring and adaptive management; IVM continuously evolves as methods are refined to achieve ecological and socioeconomic objectives (Nowak and Ballard 2005b). Within an IVM program, management objectives can be expanded beyond resistance to tree invasion. Management methods can also be designed to encourage the development of high quality habitat for pollinators (Russell et al. 2005), wildlife habitat (Clarke et al. 2006) or recreational opportunities (Nowak and Ballard 2005b). There are multiple successful examples of ecologically-based integrated vegetation management (IVM) systems that have established relatively stable plant communities and reduced economic and environmental costs of ROW management (McLoughlin 2014).

2.1.3 The IVM toolbox: vegetation management methods for power line ROWs

One of the fundamental principles of IVM is that methods need to be appropriate for site environmental and socioeconomic conditions and one method is likely not suitable for all sites (Nowak and Ballard 2005b). A range of options, therefore, must be available to managers. Prior to the 1940s, mechanical methods of brushing, mowing or hand cutting were the only tools readily available to vegetation managers, but today many strategies can be utilized.

Mechanical control of target species by brushing, mowing or hand cutting physically removes the aboveground vegetation with varying amounts of soil disturbance. Mechanical methods are still widely used across North America (Sulak and Kielbaso 2000), despite significant evidence that mowing or brushing can often increase target species reproduction and growth on ROWs (Luken et al. 1991; Yahner and Hutnik 2004). Mechanical removal of aboveground tissue encourages the growth of species that reproduce by stump or root sprouts and eventually these species assume dominance within the plant community (Ilisson and Chen 2009; Luken et al. 1991).

After the Second World War, chemical use became more common and is now widely used by many companies for power line ROW vegetation management (Sulak and Kielbaso 2000). There are many formulations and application methods for herbicide use on ROWs. Unlike mechanical mowing, herbicide applications are intended to kill both the above and belowground portions of target species (Egler 1954). Herbicides are commonly classified by how they affect the target species – i.e. their mode of action. The chemicals themselves can be broad-spectrum or selective: impacting only certain plant groups such as dicots (Stephenson and Solomon 2007). The timing of application depends on the herbicide's active ingredient and is optimized for best control at least cost. Application methods vary from non-selective (broadcast foliar spray or soil dispersal) to selective (cut stump, basal, stem-foliar and foliar) (Stephenson and Solomon 2007).

Seeding or transplanting competitive shrub and forb species - also known as ecological/biological control or manipulation – is a relatively new method with the potential to use interspecific plant competition to the manager's advantage. Specific species seeding or transplanting methods are significantly affected by local conditions and are not commonly addressed by the primary literature regarding ROWs (De Blois et al. 2004). Transplanting or encouraging natural reproduction of woody shrubs is one of two ecological manipulation strategies for ROWS. This includes layering – the process of anchoring the tips young stems to the ground to promote rooting and expansion of woody shrubs - which was found to be successful in increasing Cornus stolonifera cover on a ROW in Quebec (Meilleur et al. 1997). Coppicing - cutting main stems to encourage suckering in woody shrubs - is another enhancement method that was reported to increase stem density but not crown cover of Viburnum lentago and Cornus racemosa on New York ROWs (Ballard 2006). Seeding cover crops of competitive agronomic or native grasses is another biological manipulation strategy for disturbed sites. The seeding of Dactylis glomerata, a highly competitive grass from Eurasia, successfully established and reduced regrowth of tree transplants near Tobermory, Ontario however seeding of invasive species should be avoided (Brown 1995). Though not widely documented on power line ROWs, success with establishing native grass has been reported for abandoned gravel pits and roadsides (Maslen 1989; Tyser et al. 1998; Petersen et al. 2004). These grass communities resisted invasion of other species and persisted for multiple years.

With the large range of management tools available to today's ROW vegetation manager, there are many strategies that can alter successional trajectories of plant communities. Identifying desirable cover types for Yukon ROWs and management methods that may promote the development of these communities are discussed in the next section.

2.2 Examining vegetation management strategies to promote desired successional pathways for Yukon ROWs

2.2.1 Identifying desired cover types

Selecting an appropriate cover type for local ecological and environmental conditions is critical for limiting the growth of target species (de Blois and others 2002) and needs to be completed before potential management methods can be determined. In southern jurisdictions, maintaining or enhancing shrub cover has been identified as the most effective and logistically practical method of inhibiting target species establishment under transmission lines (Niering and Goodwin 1974; Dreyer and Niering 1986; Meilleur et al. 1994). Shade intolerance of invading tree species is frequently cited as the dominant cause of tree resistance within a shrub community (Meilleur and others 1994; Berkowitz and others 1995; Hill and others 1995). Another well documented form of tree suppression by shrubs is providing seed/seedling predator habitat. Seed predation has been demonstrated to affect the rate of tree invasion, tree species diversity, and the age structure of invading trees (Hill and others 1995; Bramble and others 1996; Ostfeld and others 1997). Predation rates are dynamic and relative to each species and their abundance. Ostfeld and others (1997) observed differences in predation rates between mice and voles, and also within their population cycles. Regardless of mechanism, shrub cover or stem density has been proven to reduce target species invasion on ROWs.

Desirable shrub species on ROWs have been documented in eastern North America (e.g. Ballard et al. 2011), but there are few shared species between eastern deciduous and northern boreal forests. Nevertheless, many northern shrubs share characteristics such as clonal growth and preference for sun exposure that have been linked to the formation of dense cover (Meilleur et al. 1994). Prickly rose (*Rosa acicularis*) and bog bilberry (*Vaccinium uliginosum*) are common shrubs on Yukon ROWs and capable of forming dense, low-growing thickets. Stable low growing shrub communities occur in many ecosystems worldwide including salmonberry (*Rubus spectabilis*) thickets in the Pacific Northwest and northern sheep laurel (*Kalmia angustifolia*) heaths in the eastern boreal forests of Newfoundland (Royo and Carson 2006). The widespread distribution of these stable shrub layers indicates a northern boreal equivalent likely exists.

In addition to shrub cover types, aggressive perennial grasses may provide a solution as they are well documented competitors of tree seedlings in the northern forestry industry (Ballandier et al. 2006). The roots and litter of bluejoint reedgrass (*Calamagrostis canadensis*), for example, can directly suppress aspen seedling and sucker development by maintaining cooler soil temperatures and physically preventing sucker penetration through the soil (Landhäusser and Lieffers 1998; Landhäusser et al. 2007). Similarly, orchardgrass (*Dactylis glomerata*) can outcompete tree species on ROWs if seeded immediately after mowing (Brown 1995). In the North, agronomic grasses seeded on a disturbed construction site above tree line limited or delayed the establishment of *Salix glauca* and *Salix alaxensis* over 11 years (Densmore 1992). Two potential cover types, therefore, may be appropriate for Yukon ROWs: a low growing shrub community or a dense mat of competitive native grass species. Which management methods may promote development of these communities remains uncertain as community development after disturbance is a site-specific and complex process.

2.2.2 Control Methods Selection

On Yukon ROWs, *Populus tremuloides, Populus balsamifera* and *Salix spp.* are the most common target species though *Betula neoalaskana* is locally dominant at wetter sites. Target species were identified as species that grow quickly after disturbance and to a height that can interfere with transmission lines. *P. tremuloides* and *P. balsamifera* are well known for aggressive suckering after aboveground disturbance (Frey et al. 2003; Ilisson and Chen 2009); willows such as *Salix bebbiana* are clonal and common in disturbed areas (Amiro and Courtin 1981; Carleton and MacLellan 1994) and *Betula* spp. are also colonizers after disturbance (Peinado et al. 1998). Mechanical control by brushing and/or mowing has traditionally been used on Yukon power line ROWs. The abundance of these target species on Yukon ROWs and their life histories strongly indicate that mechanical treatments do not promote the development of plant communities resistant to these species. Herbicide use is a potential tool for Yukon ROWs as many products are registered for the most common target species requiring management.

Many utility companies use herbicides for vegetation management on ROWs (Sulak and Kielbaso 2000). A recent review of forestry-use herbicides was completed by a consulting company, Environmental Dynamics Inc. (EDI), and aminopyralid, glyphosate, imazapyr and triclopyr were identified as candidates for use on Yukon ROWs based on their effectiveness on target species, environmental risk and use by other comparable jurisdictions (EDI 2013). A small-scale field trial by EDI indicated triclopyr and imazapyr as the most effective on northern target species. Herbicide applications for woody species control are not common in the territory and there was considerable public concern over the potential implementation of herbicide use on

Yukon ROWs. There is very little information available on herbicide behaviour in northern conditions (Newton et al. 2008) and environmental risks are difficult to estimate. To further investigate herbicides use for vegetation management on Yukon ROWs, Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr) were chosen as candidates for testing.

Triclopyr (commercial formulation Garlon XRT, 755 g L⁻¹ triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) is a pyridine-based herbicide in the carboxylic acid family. It is formulated as a butoxyethyl ether or triethylamine salt, both of which readily dissociate into triclopyr acid in water. It was first registered in Canada in 1989 as a Group 4 selective herbicide for use on broadleaf and woody vegetation in non-crop areas. Similar to the phenoxyacetic acids (e.g. 2,4-D) and benzoic acids (e.g. dicamba), triclopyr acts as an auxin mimic, effectively giving the plant a hormone overdose. As a foliar spray, triclopyr is rapidly absorbed from the leaf and translocated through the plant in as little as 12 hours (Lewer and Owen 1990). It has low leachability and the majority deposited on the forest floor remains in the organic layer (Lee et al. 1986; Thompson et al. 2000). Triclopyr typically degrades rapidly in both soil and water by microbial breakdown or photolysis (Johnson et al. 1995).

Imazapyr (commercial formulation Arsenal Powerline, 240 g L⁻¹ imazapyr acid; BASF Canada Inc., Mississauga, ON) is a broad spectrum herbicide in the imidazolinone family. It is available both as imazapyr acid or isopropylamine salt. First registered in Canada in 1994, imazapyr is a Group 2 herbicide typically used to control grasses, broad-leaf weeds and select perennial shrubs. Like the sulfonylurea family (e.g. metsulfuron), imidazolinone herbicides inhibit the production of three amino acids by binding to the acetolactate synthase (ALS) enzyme and are most effective on young, actively growing plants (Schoenhals et al. 1990). It degrades by both photolysis on the soil surface and by microbial breakdown (Wang et al. 2005; Ramezani et al. 2008). Imazapyr can be applied pre- or post-emergence and may remain active and mobile in soils for an extended period of time (Loux and Reese 1993; Bovey and Senseman 1998; Gianelli et al. 2014).

The literature strongly recommends selective herbicide treatments for preserving nontarget species, especially shrubs, as the primary method of creating tree-resistant communities (Dreyer and Niering 1986; Niering et al. 1986; Bramble et al. 1991; Mercier et al. 2001; Yahner and Hutnik 2004). The efficacy of selective herbicide on target species is also a critical component as even intact shrub communities are susceptible to invasion by suckers from established trees (Dreyer and Niering 1986). The most common selective treatments are basal bark application, cut stump/wet-blading, and targeted low-high volume foliar spray (Nowak and Ballard 2005a). Point injection has also been gaining popularity, especially for woody invasive species control (Lewis and McCarthy 2008). Non-selective herbicide treatments also change plant community structures, but generally favour annual species that do not persist long enough to inhibit target species growth (Bramble et al. 1991; Luken et al. 1994). In Alaska however, shrub control with broadcast spray applications of triclopyr resulted in higher graminoid cover as triclopyr's mode of action does not affect monocots (Seefeldt et al. 2013). Whether this grass cover persisted for more than two years or inhibited reestablishment of woody species is unknown. The complexity of vegetation dynamics after disturbance makes predictions very difficult, but by trying both selective and non-selective herbicides applied by selective and non-selective methods new community development trajectories may be induced.

Establishing graminoid cover may also be improved by direct seeding. Species selection for native grass seeding depends on species characteristics as well as site conditions and seed availability (Karim and Mallik 2008). Two prominent competitors of tree species in the boreal forestry industry, bluejoint reedgrass (Calamagrostis canadensis) and tufted hairgrass (Deschampsia caespitosa), are native to Yukon and have the potential to reduce target species regrowth under power lines (Ballandier et al. 2006). C. canadensis is a grass that aggressively develops a thick mat of roots and rhizomes and can outcompete both woody and herbaceous species for soil nitrogen (Hangs et al. 2003; Landhäusser and Lieffers 1998). In Yukon, C. *canadensis* can aggressively colonize disturbed areas where mineral soil is exposed, and stagnate ecosystem development (Simpson 2012). D. caespitosa is a slower growing grass, but once established it can successfully compete with woody species for moisture (Collet et al. 1996). Rapid colonization after disturbance is also an important characteristic for herbaceous cover crop species (Brown 1995) and slender wheatgrass (*Elymus trachycaulus*) is a rapidly establishing native grass often used for revegetation purposes (Buss et al. 1997; Petersen et al. 2004). Glaucus bluegrass (Poa glauca), violet wheatgrass (Elymus violaceus; previously E. alaskanus) and rocky mountain fescue (Festuca saximontana) are recommended for grass cover on dry sites in Yukon and are already found on Yukon ROWs (Matheus and Omzigt 2011). By applying a mix of species, graminoid covers can be designed to establish quickly, provide adequate ground cover and potentially outcompete undesirable tree and tall shrub species.

2.3 Special management considerations: phytotoxicity of herbicides to non-target plants and persistence of active ingredients in plant tissue

In northern Canada, herbicide use for woody species control is not widespread nor are its effects on northern native plant species in local soils well understood. In Alaska, applications of triclopyr and 2,4-D for shrub control had species-specific impacts on non-target vascular plants (Seefeldt et al. 2013). Forb cover overall did not decline two years after treatments, however certain species such as *Chamerion angustifolium* significantly declined in triclopyr broadcast spray plots and *Erigeron acris* was highly sensitive to 2,4-D broadcast spray. It is likely that Yukon ROW non-target plant species will also have a large range of sensitivities to herbicides. Terrestrial plant acute toxicity tests provide a standardized method to assess potential impacts on important non-target species from chemical vegetation management strategies.

2.3.1 Assessing phytotoxicity of herbicides to non-target terrestrial plants

Ecotoxicity testing to assess environmental risks of pest control products is a key component of pesticide regulation. Toxicity is the degree to which a substance causes negative effects on an organism and phytotoxicity refers to the toxicity of a substance to specifically to plants. Test organisms are subjected to a series of increasing doses and a predetermined endpoint such as surviving individuals, size or biomass is measured at the end of the test. The tests typically use non-linear regression techniques to model dose-response relationships and generate percent growth inhibition (Inhibition Concentration: IC_x) or percent mortality of individuals (Effective Concentration: EC_x) estimates (Environment Canada 2013). The estimates provide standardized values to compare toxicities of chemicals or sensitivities of organisms (Seefeldt et al. 1995). There are two tests used to characterize acute toxicity of herbicides to terrestrial plants: the vegetative vigour test and the seedling emergence and seedling growth test (OECD 2006, USEPA 1996). The vegetative vigour test evaluates sensitivity of young plants to foliar spray, while the seedling emergence and seedling growth test assesses the effect of herbicide concentrations in soil on the germination of seeds and early seedling growth. For regulatory purposes, testing is typically completed on 6-10 annual field/row crop species from multiple families with the intention of encompassing the range of any non-target plant sensitivity.

There is considerable debate whether non-target species sensitivities are adequately represented by regulatory testing (McKelvey et al. 2002; Boutin et al. 2004; Clark et al. 2004; White and Boutin 2007). Greater sensitivities of wild species than crop species to multiple

herbicides have been reported (Boutin et al. 2004), though similar sensitivities between wild and crop plants have been demonstrated as well (Carpenter and Boutin 2010; White and Boutin 2007). Within a single plant species, differences in sensitivity between cultivars and ecotypes have also been detected (White and Boutin 2007; Boutin et al. 2010). The range of sensitivities to herbicides can also vary more between wild species than agricultural ones (Olszyk et al. 2008). In addition, plant response to herbicide is also dependent on environmental conditions and even slight variations can impact sensitivity in phytotoxicity testing (Boutin et al. 2010).

To address concerns on the lack of representation of non-crop species in regulatory testing, a "List of Potential Non-Crop Species" was added as an annex to the Organisation of Economic Development and Cooperation (OECD) guidelines in 2006 (OECD 2006). Environment Canada (2013) recently released a new method for assessing the phytotoxicity of potentially contaminated boreal soils with seven boreal plant species: *Picea mariana, Picea glauca, Pinus banksiana, Populus tremuloides, Betula papyrifera, Solidago canadensis, Calamagrostis canadensis.* The use of non-crop plants presents challenges for testing, however, many argue it is essential to understand potential impacts of herbicides on non-target plants (White and Boutin 2007; Olszyk et al. 2008; Boutin et al. 2012).

The production of homogenous "crops" of wild plants for toxicity testing is a significant challenge. Seed for wild species is less readily available than for field/row crops (White et al., 2009) and quality is less consistent (Pallett et al. 2007). Many wild species' seeds also have dormancy requirements that must be met to maximize germination percentages (White et al., 2009). Wild plants are often slower to reach the required growth stage for testing (Boutin et al. 2004) and there is higher intrinsic variability in individual plant growth rates and biomass (Pallett et al. 2007). Nevertheless, there are successful examples of wild plant species meeting regulatory criteria for valid toxicity testing (Boutin et al. 2004; Olszyk et al. 2008; Boutin et al. 2010; Princz et al. 2012).

2.3.2 Benefits of toxicity with ecologically relevant species and substrates

Estimating boreal plant species sensitivities to herbicides is difficult as there is limited background information on herbicide behaviour in northern Canada. It is uncertain whether native boreal species are similarly sensitive to herbicides as crop species. Princz et al. (2012), for example, found boreal plants to be more sensitive to hydrocarbon contaminated soil than crop species but similarly sensitive to soil salinity. Shrub control research in Alaska indicates

herbicide sensitivities of boreal plants are species dependent and sometimes site specific (Seefeldt et al. 2013). The sensitivities of the two most frequent and abundant native herbaceous species at the Yukon ROW research sites, *Achillea millefolium* and *Chamerion angustifolium*, are of particular concern. *A. millefolium* and *C. angustifolium* are widespread rhizomatous perennials that are ecologically and culturally important. Snowshoe hare (*Lepus americanus*), a keystone boreal species, feeds on both plant species during the late summer (Seccombe-Hett and Turkington 2008). *C. angustifolium* is also particularly attractive to bees and other pollinators (Kevan et al. 1993) and is an important component of moose summer diet (Johanson et al. 1994). Both species are culturally important as edible and/or medicinal plants (Gray 2012). Covering more than 5% of Yukon ROW research sites, the disappearance *A. millefolium* and *C. angustifolium* and *C. angustifolium* may have negative effects on ROW plant communities and ecosystems. The use of these two species for phytotoxicity testing provides species-specific toxicity information to increase knowledge of herbicide impacts on non-target plants on Yukon ROWs.

In addition to species variability, bioavailability of herbicide can differ depending on soil characteristics (Loux and Reese 1993; Eliason et al. 2004; Allison et al. 2013). The sorption of herbicide to soil particles can lower the amount of herbicide readily absorbed by plant roots and thus, toxicity to seeds and seedlings may be site/soil specific. Triclopyr and imazapyr are weak acids and exist mostly in their anionic state in all but the most acidic soils (Johnson et al. 1995; Gianelli et al. 2014). With a negative charge, these chemicals do not sorb strongly to soil particles and are relatively mobile. When deposited on the forest floor, the majority of triclopyr residues remain in the organic soil horizon (Thompson et al. 2000) suggesting triclopyr sorption will increase with organic carbon content. Imazapyr does not readily sorb to organic matter unless soil pH is very low (<5) and bioavailability is not typically affected by soil organic carbon (Pusino et al. 1997). Imazapyr sorption is positively associated with clay, iron and aluminum content and imazapyr is likely less available to plants in clay, iron and /or aluminum rich soils (Gianelli et al. 2014). Both herbicides are degraded in soil primarily through microbial breakdown (Johnson et al. 1995; Gianelli et al. 2014) and the use of native soils with intact microbial communities better represents field conditions than sterilized soil. The use of field collected soils for seedling emergence and seedling growth tests provides a better representation of northern ROW conditions than generic potting soil and incorporates the effects of soil type on herbicide bioavailability into the test.

2.3.3 Persistence of herbicide active ingredient in plant tissue

In addition to acute phytotoxicity of herbicides to plants, the dissipation rates of these chemicals in plant tissue is also of concern. As the primary producers in intricately connected ecosystems, grasses, forbs and shrubs provide pathways for herbicide residue into the wider environment including transfer to wildlife (Tatum 2004). Foliage can also act as a source for soil contamination when fallen leaves decompose on the forest floor (Thompson et al. 1994). It is widely accepted that the rate of dissipation of herbicides from vegetation is significantly dependent on environmental conditions (Newton et al. 2008); how northern climates will impact dissipation rates is not well understood.

If the herbicide persists on the leaf surface, it can dissipate through volatilization, photolysis or microbial breakdown (Bentson and Norris 1991; Newton et al. 2008). The net effect of northern environmental conditions on these processes is unknown. Long photoperiods in the summer associated with higher latitudes may increase the rate of photolysis on the leaf before it can be absorbed, however, microbial breakdown may be slower due to cooler temperatures. Once absorbed, degradation requires it to be metabolized or deposited as foliage (Newton et al. 1990). The ability to metabolize herbicides is species specific as demonstrated by Sidhu and Feng (1993). Plant metabolic activity is limited in the North partially due to cool soil temperatures (Bonan and Shugart 1989) and this may increase the residency of herbicide in plant tissue in the North. Temperature was identified as a major factor influencing dissipation rate from foliage by Newton et al. (1990). Triclopyr rapidly dissipated from foliage within the first 80 days after application, but concentrations within vegetation changed very little over the winter. In Alaska, however, Newton and others (2008) found dissipation rates of triclopyr and imazapyr from vegetation similar to those reported at more southern latitudes. The strong influence of environmental conditions on the dissipation of herbicide from plant tissue suggests more research is needed to confirm whether rates are similar to southern regions.

2.4 References

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Preamble: Chapter 3

The first data chapter explores the impacts of eight ROW vegetation management treatments on plant species and communities. Research in other jurisdictions indicates ROWs can be managed to promote low growing vegetation that naturally inhibit reinvasion of target species. This is typically accomplished with herbicide applied selectively to target species. A field experiment was installed at four sites in Yukon and vegetation cover data and herbicide damage assessments were conducted. In this chapter, the control of target species is evaluated for each management method. Damage assessments and percent cover changes provide additional information on impacts to non-target species. Knowledge of non-target responses to treatments is critical when selecting appropriate management methods for ROWs. This value of small-scale testing confirmed by the discovery of chlorosis and deformity of non-target plants one year after imazapyr treatments, even when the herbicide was only applied to target species. The damage to non-target plants differed in severity by species and highlights the need for more focused toxicity testing to better understand potential impacts of herbicide applications on Yukon ROWs.

3.0 EARLY VEGETATION RESPONSES TO EIGHT INTEGRATED VEGETATION MANAGEMENT TECHNIQUES ON NORTHERN RIGHTS-OF-WAY

3.1 Abstract

Integrated vegetation management programs have successfully reduced the frequency and intensity of power line right-of-way management by promoting low growing plant communities resistant to tree invasion. To examine whether these principles are transferable to northern ecosystems, we tested eight treatments at four sites in Yukon, Canada. Two herbicides, imazapyr and triclopyr, were applied by three methods, as well as a native grass seeding treatment and a mowing control. Vegetation cover was recorded prior to treatment and after one year along with herbicide damage assessments. Overall, treatments caused significant changes to vascular plant communities after one year. Short-term control of woody target species was greater in chemically treated plots (66-94%) than with mechanical methods (<55%). All treatments caused a minor reduction in non-target vegetation cover. In seeded plots, seedlings emerged but total non-target species cover was reduced by seedbed preparation. Triclopyr broadcast spray reduced non-target vegetation cover by <10%, but the common shrub, kinnikinnick (Arctostaphylos uvaursi), was highly impacted. Selective application of triclopyr effectively controlled targets with minimal effects on non-target species. Imazapyr consistently caused more impacts to non-target plants than triclopyr. Both selective and non-selective imazapyr applications resulted in chlorosis, stunting and deformity of shrubs and forbs one year after treatment. This suggests imazapyr can remain active in northern soils for at least 365 days as well as transfer to untreated plants. The range of sensitivities of boreal plant species to imazapyr and triclopyr and potential persistence in northern soils highlights the need for focused toxicity research in the North.

3.2 Introduction

Electrical utility rights-of-way (ROWs) present demanding challenges for vegetation management as safe, reliable electrical service is compromised by trees near or underneath transmission lines. An extensive range of mechanical, chemical and biological methods are available to vegetation managers and allowing for more complex management regimes. Rather than simply "resetting" succession to a previous stage, management methods can be designed to alter the plant species and abiotic conditions on a ROW and fundamentally change the direction of plant community development (Rosenberg and Freedman 1984; Niering 1987; Pickett et al.

2009). Certain low-growing plant communities or "cover types" have been proven to reduce tree establishment on ROWs and strategic management can facilitate their development (Bramble et al. 1991; Meilleur et al. 1994; Yahner and Hutnik 2004; McLoughlin 2014). Integrated Vegetation Management (IVM) encompasses the systematic approach of understanding and manipulating ROW plant communities to meet management objectives with minimum cost and environmental impact (Nowak and Ballard 2005).

A key component of IVM is understanding local vegetation dynamics, especially identifying low growing plant communities that resist the regrowth of trees and the techniques that encourage the formation of such communities (Niering 1987; Nowak and Ballard 2005). In southern jurisdictions, maintaining or enhancing shrub cover is the most effective method of inhibiting target species establishment; this objective is typically achieved by selective herbicide application to individual tree and tall shrub stems or foliage Niering and Goodwin 1974; (Dreyer and Niering 1986; Bramble et al. 1991; Meilleur et al. 1994; Yahner and Hutnik 2004). A modern example of IVM successfully promoting shrub communities exists in New York State, where the use of selective herbicide applications on power line ROWs has been mandated since the 1980s (McLoughlin 2014). Whether IVM principles and selective herbicide techniques will produce similar results in northern boreal ecosystems has not been tested.

The Yukon Territory, in northern Canada, is dominated by boreal forests and has over 1000 km of power line ROWs that have traditionally been cleared by mowing and brushing. The use of herbicide for woody plant control is not common in the area and knowledge of the effectiveness and non-target impacts of herbicides under local conditions is lacking. In addition, it is unclear how herbicide dissipation rates will be affected by the cold climate. Herbicide degradation is often a function of temperature; however there is evidence that northern soil microbes can metabolize herbicides at lower temperatures than reported elsewhere (Newton et al. 2008). Herbicide use is a potential tool for Yukon ROWs as many products are registered for the most common target species requiring management: trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), and willows (*Salix* spp.). Aspen and poplar are well known for aggressive suckering after aboveground disturbance (Frey et al. 2003; Ilisson and Chen 2009); willows such as *Salix bebbiana* are clonal and common in disturbed areas (Amiro and Courtin 1981; Carleton and MacLellan 1994). The abundance of these target species on Yukon ROWs and their life histories strongly indicate that mechanical treatments do not promote the

development of plant communities resistant to these species. It is difficult to predict which treatments and subsequent cover types may be most advantageous because to our knowledge there are no examples of long term IVM programs in northern boreal regions.

Desirable shrub species on ROWs have been documented in eastern North America (e.g. Ballard et al. 2011), but there are few shared species between eastern deciduous and northern boreal forests. Nevertheless, many northern shrubs share characteristics such as clonal growth and heliophily that have been linked to the formation of dense cover (Meilleur et al. 1994). Stable low growing shrub communities occur in many ecosystems worldwide (Royo and Carson 2006) and it is likely a boreal equivalent exists.

In addition to shrub cover types, aggressive perennial grasses may provide a solution as they are well documented competitors of tree seedlings in the northern forestry industry (Ballandier et al. 2006). The roots and litter of bluejoint reedgrass (*Calamagrostis canadensis*), for example, can directly suppress aspen seedling and sucker development by maintaining cooler soil temperatures and physically preventing sucker penetration through the soil (Landhäusser and Lieffers 1998; Landhäusser et al. 2007). Similarly, orchardgrass (*Dactylis glomerata*) can outcompete tree species on ROWs if seeded immediately after mowing (Brown 1995). Exploiting natural competitive interactions by planting aggressive native grasses may be facilitated through selective herbicide applications, direct seeding or a combination of both.

The objectives of this study were, 1) to assess the effectiveness of eight ROW management treatments, including selective and non-selective herbicide applications and native grass seeding, 2) evaluate the impacts of herbicide applications on non-target vegetation, and 3) examine treatment induced changes to plant community composition and structure.

3.3 Material and Methods

3.3.1 Study Area

Four study sites were located on power line ROWs in Yukon, Canada. Sites were distributed across the territory within the Boreal Cordillera Ecozone and were representative of the ecotypes where ROWs are found (Table 3.1.). Sites were selected for the study by aerial survey to ensure homogeneity of vegetation type and similar development since the last mowing cycle which had occurred between one and six years previously. The three more southern sites (CAR, HJ1 and HJ2) were bordered by mid-successional boreal forests dominated by white spruce (*Picea glauca*) and trembling aspen (*Populus tremuloides*) and the more northern DAW

site bisected a mature coniferous stand (*Picea* spp.). Dominant vascular plant covers on the ROWs prior to application are summarized in Table 3.2.

Table 3.1. Location and description of right-of-way vegetation management study sites in Yukon. Climate data is from Environment Canada Climate Normals (1981-2010) for Mayo Road (CAR), Dawson Airport (DAW) and Otter Falls (HJ1 and HJ2). Soil classification was derived from White et al. (1992).

Site	Coordinates	Ecoregion	Mean Annual Precip. (mm)	Mean January Temp (°C)	Mean July Temp (°C)	Soil Type	Year of Last Mowing Treatment
CAR	61.8° N, 136.0° W 61.9°N, 136.1°W*	Yukon Plateau - Central	323.4	-17.2	14.9	Eutric Brunisol on Sand	2010
DAW	63.9°N, 138.4°W	Yukon Plateau - North/ Klondike Plateau	324.3	-26.0	15.7	Eutric Brunisol on Sand	2008
HJ1	60.8°N, 136.6°W	Yukon Southern Lakes	297.3	-16.1	13.0	Eutric Brunisol on Clay Loam	2013
HJ2	60.8°N, 136.0°W	Yukon Southern Lakes	297.3	-16.1	13.0	Eutric Brunisol on Clay Loam	2011

*The CAR site consisted of two blocks at one access, and one block 10 km north to avoid surface water drainages

Yukon's climate is classified as subarctic continental with precipitation at lower elevations ranging from 250-300 mm annually (Smith et al 2004). Weather conditions for each site were obtained from the nearest Yukon Wildland Fire Management stations and compared to thirty-year Environment Canada Climate Normals (1981-2010) for the regions (Table 3.3.). Overall, conditions were within normal ranges though above average May temperatures occurred across the territory in 2015 and resulted in an early spring. The DAW site experienced more precipitation than normal both seasons, but the elevation difference between the Antimony and Dawson A stations (~170 m) likely contributed to the difference; precipitation typically increases with elevation in Yukon (Smith et al 2004).

Site	Dominant Species in 2014	Average % Cover of Site Before Treatment	Site	Dominant Species in 2014	Average % Cover of Site Before Treatment
CAR	Picea glauca	18.4	HJ1	Populus tremuloides	6.4
	Populus tremuloides	14.2		Arctostaphylos uva-ursi	37.0
	Rosa acicularis	6.1		Chamerion angustifolium	4.4
	Linnea borealis	8.7		Calamagrostis purpurascens	7.8
	Chamerion angustifolium	7.0		Bromus pumpellianus	3.7
	Calamagrostis purpurascens	13.3			
			HJ2	Populus tremuloides	17.4
DAW	Betula neoalaskana	7.3		Salix spp.	13.9
	Salix spp.	13.9		Shepherdia canadensis	11.6
	Vaccinium uliginosum	10.0		Arctostaphylos uva-ursi	10.2
	Rhododendron groenlandicum	8.0		Fragaria virginiana	10.4
	Vaccinium vitis-idaea	6.1		Calamagrostis purpurascens	7.8
	Cornus canadensis	11.1			
	Chamerion angustifolium	6.0			
	Festuca altaica	7.7			

Table 3.2. Average percent cover of dominant vegetation on four Yukon ROW sites prior to treatment in 2014
Table 3.3. Average summer daily temperatures and total precipitation during the 2014/2015 study period near four Yukon ROW study sites in comparison to 1981-2010 climate normals (Environment Canada n.d.) for each region.

				A	verage	Daily T	empera	ature (°C	C)	Av	verage 7	Fotal Pr	recipitat	tion (m	m)
Data Source	Station	Coordinates	Elevation		2014			2015			2014			2015	
				Jul.	Aug.	Sep.	May	Jun.	Jul.	Jul.	Aug.	Sep.	May	Jun.	Jul.
Yukon Wildland Fire	Antimony	64.0° N	544	15.0	12.0	5.2	13.0	14.0	14.6	53.4	59.8	36.6	61.2	60.5	81.0
Management	1	138.6° W	011	10.0	12.0	0.2	, 15.0	11.0	1	55.1	27.0	2 5.0	01.2	00.0	0110
Environment Canada 1981-2010	Dawson A	64.0° N	370	15.7	12.3	5.8	8.2	14.0	15.7	49.0	43.4	34.0	30.8	38.2	49.0
Normals	Dawsonn	139.1° W	570	10.7	12.0	5.0	0.2	1110	10.7	12.0		0.110	50.0	00.2	.,,,,,
Yukon Wildland Fire	Braeburn	61.º5 N	725	14.6	123	60	11 1	13.0	14.0	47.8	30.6	64 8	167	43 5	66.2
Management	Drucoum	138.8° W	11.0	12.5	0.0	11.1	15.0	11.0	17.0	50.0	01.0	10.7	15.5	00.2	
Environment Canada 1981-2010	Mayo Road	60.9° N	655	14.9	12.8	71	77	12.9	14 9	51.0	47.9	35.9	25.3	39.0	51.0
Normals	Wayo Koau	138.2° W	17.7	12.0	/.1	/./	12.7	14.7	51.0	77.7	55.7	25.5	57.0	51.0	
Yukon Wildland Fire	Champagne	60.8° N	756	1/3	12.5	67	7 11 2	12.5	1/1 3	52.0	34.0	08.8	8.4	20.4	31 /
Management		136.4° W		14.5	12.5	0.7	11.2	15.5	14.5	52.0	54.0	90.0	0.4	59.4	51.4
Environment Canada 1981-2010	Ottor Follo	61.0° N	830	13.0	10.0	50	5.0	10.0	12.0	515	13 1	21.0	21.0	127	515
Normals	Ouer Fails	137.1° W 850	13.0	10.8	5.0	5.9	10.9	13.0	54.5	43.1	51.0	21.0	43.7	54.5	

3.3.2 Sampling Design

Each of the four sites was laid out in a randomized complete block design. At each site three blocks with eight randomly assigned treatment plots per block were installed. The 6 m x 6 m treatment plots were spaced at a minimum of 50 m apart to avoid interference between treatments (i.e., herbicide drift). Four 1 m² permanent vegetation cover plots were established within each treatment plot and percent cover data recorded 5-14 days before treatments were applied in July of 2014. Total percent cover of each species was recorded to the nearest percent and all unknown species were collected from outside the plot for later identification. Vegetation cover was recorded again in 2015 within ten days of the original observation dates.

Eight treatments were designed to represent mechanical, chemical and biological strategies for ROW vegetation management (Table 3.4.). The control was mechanical mowing, the current standard treatment, which was simulated by hand cutting all vegetation at 10-20 cm above the soil surface. Two common products used for woody species control were selected: Garlon XRT (755 g L⁻¹ triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) and Arsenal Powerline (240 g L⁻¹ imazapyr acid; BASF Canada Inc., Mississauga, ON). These two herbicides were applied through three methods: broadcast spray, cut stump and point injection at the maximum rates specified on the labels (broadcast spray: 4530 g a.i. ha⁻¹, 720 g a.i. ha⁻¹; cut stump and point injection: 143.5 g a.i. L⁻¹ canola oil, 22.6 g a.i. L⁻¹ DI water). A backpack sprayer was used for the broadcast spray treatment. Cut stump applications were completed by hand cutting all vegetation at 20-30 cm and applying products to all cut stems with a paint brush. Point injections were applied via a syringe inserted into a small drilled hole or incision in the stem of a target species. In selective cutting plots, only target species were hand cut and removed. Point injection and selective cutting plots were also seeded with native grasses at 50 kg ha⁻¹ as high seeding rates have been shown to reduce species invasion of disturbed areas in Yukon (EDI 2009). Litter was raked out of the plot to prepare the seed bed and a native grass seed mix of 42% (b/wt) violet wheatgrass (Elymus violaceus), 26% slender wheatgrass (Elymus trachycaulus), 8% rocky mountain fescue (Festuca saximontana), 6% glaucous bluegrass (Poa glauca), 5% bluejoint reedgrass (Calamagrostis canadensis) and 2% tufted hairgrass (Deschampsia caespitosa) from DLF Pickseed Canada (Lindsay, ON) was broadcast by hand. After seeding the plot was lightly raked to ensure good seed-soil contact. Treatments were applied between mid-July and early August 2014.

Treatment	Abbreviation	Strategy	Description
Mowing (Control)	МС	Mechanical	Cut and removed all vegetation at 10-20 cm above soil surface
Broadcast Spray - Triclopyr	BS-T	Chemical	Applied herbicide with a backpack sprayer to all
Broadcast Spray - Imazapyr	BS-I	Chemical	vegetation; any stems above 1.5 m were cut prior to spraying
Cut Stump - Triclopyr CS-T C		Chemical	Cut all vegetation at 20-30 cm above soil surface and
Cut Stump - Imazapyr	CS-I	Chemical	applied herbicide with a paintbrush
Point Injection - Triclopyr	PI-T	Chemical/Biological	Incised small stems/drilled large stems of targets
Point Injection - Imazapyr	PI-I	Chemical/Biological	native grasses
Selective Cutting	SC	Mechanical/Biological	Hand cut and removed targets; seeded native grasses

Table 3.4. Description of eight right-of-way vegetation management treatments applied in four sites within Yukon in 2014.

Target species were defined based on two criteria: rapid regrowth after disturbance and the ability to grow tall enough to interfere with transmission lines. Trembling aspen, balsam poplar and willows were present at every site and Alaska paper birch (*Betula neoalaskana*) was included at the DAW site. While conifers in the Yukon Territory can grow to a height where they may interfere with lines, due to their very slow growth rates, conifers are not considered a management concern by the utility company and were thus not included as target species.

Visual herbicide damage assessments were completed one year after application in all chemically treated plots. Targets were evaluated by species and damage to treated stems and new suckers/seedlings were separated to identify duration of effect. Non-targets were assessed by life form: erect shrubs (<1.5 m in height), prostrate shrubs, forbs and graminoids. A scale of 0-100 was used with 0 being unaffected and 100 being completely dead. Only herbicide-related damage was recorded and untreated areas surrounding the plot were used as a reference to differentiate between natural and herbicide damage.

Species richness and evenness were determined for each treatment plot using the average cover and total number of species from the four vegetation cover subplots. Species richness was defined as the total number of species per plot and evenness was calculated with the EVar index based on the average percent cover of each species per plot (Smith and Wilson 1996).

3.3.3 Statistical Analysis

Treatment effects on the responses of target and non-target species, species richness, and species evenness were analyzed via linear mixed-models using the R library "lmerTest" (Kuznetsova et al. 2014). Assumptions of normality and equal variance were checked post hoc

with QQ plots and fitted vs. residual plots. If significant ($\alpha = 0.05$), differences between least squared means of each factor combination were generated by function "difflsmeans" and sorted to assess differences within factors.

Efficacy of target species control by treatment was assessed by converting 2014 and 2015 cover data into percent control: [(2014 cover – 2015 cover)/2014 cover] * 100. An increase in cover post-treatment was truncated at 0 for "no control of target species". Data points with <1% cover of a target species in 2014 were removed from the analysis as even the most marginal change generated large percent control values and dominated the analysis. Prior to modelling, percent control data were power transformed (λ =2) to stabilize the variance and meet normality assumptions of linear mixed-models. Treatment, species and their interaction were fixed factors with site and block as random variables.

Non-target species abundances were grouped by life form: erect shrubs, prostrate shrubs, forbs and graminoids. Conversion to proportions of 2014 cover overemphasized small changes by life forms with minimal cover. For example, life forms with 3% cover in 2014 and 2% cover in 2015 would have decreased by ~30% which exaggerates the change's significance. The absolute difference in cover between 2014 and 2015 was thus selected as the response variable for non-target species to better represent the magnitudes of changes. Treatment, life form and their interaction were included as fixed effects and site and block were random variables.

To test for herbicide by application type interactions, treatments were separated into herbicide and application type factors. Zero to one hundred damage values were log(x+1) transformed prior to analysis as the distribution was log normal and to meet assumptions of equal variance and normality of model residuals. Target and non-target vegetation were again modelled separately. Damage analysis for target species included four main factors (age, herbicide, application type and species) and all potential interactions. Age accounted for the difference between treated stems and newly sprouted seedlings or suckers. The model for non-targets tested herbicide, application type, life form and their interactions as fixed factors. Both damage assessment models included site and block as random variables.

The species richness and evenness models included treatment, site and their interaction as fixed factors and block as the random variable. Data from before treatment and one year after were modeled separately. Community changes following treatment were explored using non-metric multidimensional scaling ordination (NMDS) (McCune and Grace 2002). NMDS does not require

assumptions of linear distributions within the data and is robust against differences in beta diversity. The ordination was conducted in R library "vegan" with Bray-Curtis distances using function "metaMDS" for automated testing of dimensions and best fit (Oksanen et al. 2015). Tests for treatment effects on community composition one year after treatment were made using PERMANOVA with Bray-Curtis distances in R library "vegan" (Oksanen et al. 2015). PERMANOVA is a non-parametric multivariate analysis of variance using distance matrices that can incorporate complex experimental designs (Anderson 2001). Treatment, site and a treatment:site interaction were fixed effects in the analysis with species abundance data one year following treatment as the response. The analysis was stratified by block with 999 permutations. The assumption of similar dispersion was checked post-hoc and both treatment and site data were within acceptable ranges (p values of 0.67 and 0.06).

All statistical analyses were completed in R version 3.1.2 (R Core Team 2015).

3.4 Results

3.4.1 Control of Target Species

Control of target species one year following treatment was greater in most chemically treated plots than after selective cutting and mechanical mowing (ANOVA, $F_{7,118}$ =4.29, p <0.01). Imazapyr broadcast spray provided the greatest control and mowing was the least effective (Figure 3.1.). One target species was not more sensitive to treatments than others ($F_{3,115}$ =0.67, p=0.93) and there was also no species by treatment interaction ($F_{20,118}$ =0.91, p=0.50). Imazapyr was only more effective than triclopyr when applied by broadcast spraying (94% ±1.7SE, n=21 vs. 82% ±7.6SE, n=17, where n are treatment plots) and both herbicides provided equivalent control in cut stump and point injection plots. Cut stump with triclopyr was the least effective chemical treatment and was not different from selective cutting.



Figure 3.1. Control of target species on Yukon ROWs one year after eight vegetation management treatments. Percent control is defined as the difference in cover between 2014 and 2015, divided by 2014 cover x 100. Shading indicates type of herbicide and treatment codes are described in Table 3.4. Error bars represent standard error with n= 17, 21, 19, 16, 22, 26, 16, 15 for each treatment; n differs between treatments as all four target species were not present in each plot. Different letters indicate statistically significant differences between least square means (p <0.05).

In general imazapyr caused more visual herbicide damage to target species than triclopyr; however this difference was only measured in damage to new seedlings and suckers. Mean damage values to the previous year's treated stems by imazapyr and triclopyr were 95 ±1.2SE, n=74, and 95 ±1.5SE, n=76, (out of 100) respectively, whereas growth in imazapyr plots had more residual herbicide damage than triclopyr (Table 3.5.). Damage was greater for plants that were directly treated than for those that emerged the following growing season (Age). Directly treated birch (98 ±1.0SE, n=16) and aspen (98 ±1.2SE, n=56) were more damaged than poplar (93 ±2.9SE, n=30), but similar to willows (93 ±1.8SE, n=48). Damage to new growth was comparable among most species and the only difference was greater damage to willows (13 ±3.6SE, n=33) compared with aspen (10 ±2.1SE, n=54).

Table 3.5. Linear mixed-model summaries for herbicide damage (0-100) to target species and nontarget vegetation one year after right-of-way vegetation management treatments. Non-target life forms were erect shrubs (<1.5 m high), prostrate shrubs, forbs and graminoids. Both models tested herbicide (H), application type (AT), species or lifeform (SP/LF), and all interactions as factors. Age (AG) was also tested as a factor influencing damage to target species and had two levels: directly treated in 2014 or newly sprouted seedling/sucker in 2015. Models included site and block as random variables.

Source	Target Species	Non-Target Life Forms
Age (AG)	F _{1,220} =1726.51, p<0.01	
Herbicide (H)	F _{1,224} =14.89, p<0.01	F _{1,214} =213.91, p<0.01
Application Type (AT)	F _{2,224} =1.39, p=0.25	F _{2,217} =43.79, p<0.01
Species/Life form (SP/LF)	F _{3,226} =1.41, p=0.23	F _{3,215} =22.24, p<0.01
AG x H	F _{1,218} =15.15, p<0.01	
AG x AP	F _{2,220} =0.96, p=0.39	
AG x SP	F _{3,220} =3.97, p<0.01	
H x AP	F _{2,221} =1.65, p=0.19	F _{2,214} =0.97, p=0.38
H x SP/LF	F _{3,224} =1.34, p=0.26	F _{3,214} =5.89, p<0.01
AP x SP/LF	F _{6,224} =2.01, p=0.07	F _{6,214} =3.26, p<0.01
AG x H x AT	F _{2,220} =2.06, p=0.13	
AG x H x SP/LF	F _{3,220} =0.16, p=0.92	
AG x AT x SP/LF	F _{6,221} =0.57, p=0.75	
H x AT x SP/LF	F _{6,223} =0.37, p=0.90	F _{6,214} =9.08, p<0.01
AG x H x AT x SP/LF	F _{6,220} =0.93, p=0.47	

3.4.2 Response of Non-Target Vegetation

Treatments caused significant changes in non-target vegetation cover (ANOVA, $F_{7,314}=5.47$, p<0.01). Most treatment applications resulted in a neutral or negative change after one year. Treatments rarely caused cover changes greater than ±10% of the plot area and no distinct trends across vegetative life forms (erect shrubs, prostrate shrubs, forbs and graminoids) were detected. Treatment effects on cover change were life form specific as demonstrated by a very strong interaction among life form and treatment ($F_{21,314}=2.74$, p<0.01; Figure 3.2.). Differences between treatment means and zero are listed in Table 3.6. Visual herbicide damage assessments indicated imazapyr was more damaging than triclopyr, with main effects of herbicide, application type, life form and most two-way interactions significant (Table 3.5.). In non-target species, damage by application type was consistent with the selectivity of the method: broadcast spray caused more damage followed by cut stump and point injection (Figure 3.3.). Life form was a significant factor in explaining visual damage with erect shrubs being the most sensitive.



Figure 3.2. Change in non-target vegetation cover by life form one year after eight ROW vegetation management treatments in Yukon. Bars represent the difference in percent cover between 2014 and 2015. Shading indicates type of herbicide applied and treatment codes are described in Table 3.4. Error bars represent standard error and for erect shrubs: n= 11,9,10,8,9,10,8,12 and prostrate shrubs: n=11,11,12,10,10,11,11,11. For both forbs and graminoids n=12 across all plots. Different letters indicate statistically significant differences between least square means (p<0.05).

Treatment	Erect Shrubs	Prostrate Shrubs	Forbs	Graminoids
BS-T	Yes	Yes	Yes	No
BS-I	Yes	No	Yes	Yes
CS-T	No	Yes	Yes	No
CS-I	Yes	No	Yes	Yes
PI-T	No	No	No	No
PI-I	No	Yes	Yes	Yes
SC	No	No	Yes	No
MC	No	No	No	Yes

Table 3.6. List of mean percent cover changes of non-target life forms statistically different from zero. "Yes" indicates means are different from zero based on no overlap between each mean's 95% confidence intervals and zero. Treatment codes are described in Table 3.4.



Figure 3.3. Herbicide damage to non-target vegetation one year after eight ROW vegetation management treatments in Yukon. Mean damage assessment values (0-100) are grouped by life form and shading indicates the different herbicides used. Treatment codes are described in Table 3.4. Error bars represent standard error and for erect shrubs: n=11,7,9,6,8,10; prostrate shrubs: n=11,12,11,9,10,10; forbs: n=12,12,11,11,11,11; and graminoids: n=12,12,11,11,11,11.

Erect shrub cover was reduced one year after treatment in all plots except triclopyr point injection and selective cutting. The impacts of chemical treatments were dependent on herbicide type. Broadcast spray treatments resulted in similar decreases in cover of erect shrubs, but visual damage by imazapyr was greater (Figure 3.3.). Cut stump with imazapyr treatments reduced plot cover by more than 15% and damage was high ($62 \pm 10.13SE$, n=6), but only minor cover reduction and phytotoxic effects were measured in triclopyr cut stump plots. Though point injection treatments were only applied to target species, non-target erect shrub cover was affected by the type of herbicide: cover increased in point injection with triclopyr plots (+ $5.53\% \pm 5.4SE$, n=9) and decreased in imazapyr point injection plots (- $5.7\% \pm 3.1SE$, n=10). This was consistent with damage assessments; triclopyr point injection caused almost 0 visual damage compared to imazapyr (27.5 ± 7.19 SE, n=10). As imazapyr was not point injected into erect shrubs, transfer was occurring by an unknown belowground mechanism. There was no change in erect shrub cover in selective cutting plots and mowing treatments resulted in a minor decrease of 2.4% ($\pm 0.9SE$, n=12).

Prostrate shrub cover did not change substantially after most treatments with the exception of broadcast spray with triclopyr, which reduced cover by 15.9% (\pm 6.2SE, n=11). In contrast, prostrate shrubs were only slightly impacted by imazapyr spray (-2.6% \pm 2.3SE, n=11) and damage assessment values were also low (15 \pm 2.61SE, n=12). Cut stump treatments caused a weak (<5%) increase in cover regardless of herbicide type and damage from herbicide was limited (<10). Selective cutting and mowing both resulted in reduced prostrate shrub cover of ~2%.

Forb cover decreased across all treatments with declines ranging from -2.2 to -12.0%; there were few differences between treatments (Figure 3.2.). Visual herbicide damage was more evident in imazapyr than triclopyr plots regardless of application type. Both broadcast spray treatments reduced cover by ~10%. Cut stump plots also caused a similar decrease in cover of -8.0% (\pm 2.2SE, n=12) and -5.3% (\pm 3.4SE, n=12). Forb cover decreased slightly in both point injection treatments with means of -2.2% \pm 2.1SE, n=12 (triclopyr) and -3.5% \pm 1.7SE, n=12 (imazapyr). Selective cutting caused a reduction in cover similar to all other treatments and a minor decrease in forb cover was also measured in mowing plots.

Triclopyr's mode of action targets dicots and thus graminoid cover was not affected by triclopyr treatments. Imazapyr reduced graminoid cover with broadcast spray and cut stump applications resulting in similar changes of -11.0 (\pm 1.2SE, n=12) and -10.2% (\pm 3.7SE, n=12) and

point injection plots showing a 5.7% (± 2.3 SE, n=12) decrease in cover. Selective cutting resulted in <2.0% reduction in cover and mowing decreased graminoid cover by 4.9% (± 2.1 SE, n=12). Damage assessments were consistent with the cover data: only imazapyr treatments caused substantial damage. Imazapyr broadcast spray was the most damaging (59 ± 8.2 SE, n=12), followed by cut stump (9 ± 5.4 SE, n=11) and point injection (8 ± 2.9 SE, n=11).

3.4.3 Vascular Plant Community Change

Differences in plant communities were observed among sites with the DAW site being the most distinct (Figure 3.4.). Based on species scores, NMDS Axis 1 represents a gradient between a dry, grassy understory of purple reedgrass (*Calamagrostis purpurascens*), Pumpelly's brome (*Bromus pumpellianus*) and glaucous bluegrass (*Poa glauca*) to a moist ericaceous community dominated by bog bilberry (*Vaccinium uliginosum*) and Labrador tea (*Rhododendron groenlandicum*) (Appendix 1). The CAR, HJ1 and HJ2 sites overlap along NMDS Axis 1 indicating the presence of similar, drought tolerant grasses that were not common at the wetter, shrubby DAW site. NMDS Axis 2 represents an increase in prostrate shrub cover (i.e. lowbush cranberry (*Vaccinium vitis-idaea*) and kinnikinnick (*Arctostaphylos uva-ursi*) and a corresponding decrease in forb cover (e.g. anemone species (*Anemone* spp.), wild strawberry (*Fragaria virginiana*), and tall lungwort (*Mertensia paniculata*)). The abundance of prostrate shrubs, especially kinnikinnick, distinguishes the HJ1 site from HJ2 along NMDS axis 2. Differences in plant communities between sites was further confirmed by PERMANOVA (Site: F₃=14.78, p<0.01).



Figure 3.4. NMDS ordination of 2014 and 2015 species abundance data from each site: (• = CAR, \blacktriangle =DAW, • = HJ1, \square = HJ2). The NMDS identified a two dimensional solution after 100 iterations with a final stress of 0.22. Axis NMDS1 represents a gradient from drought tolerant to moisture loving species. Increasing NMDS2 scores are associated with a transition from sites with a greater abundance of forbs to sites with increasing abundance of lowbush cranberry and kinnikinnick. Segments connect plots in 2014 to the same plots in 2015, indicating the magnitude and direction of vegetation changes one year after treatment. The magnitude of change in the vegetation community between years varies by plot and is indicated by short (limited change) or longer (greater change) segments. The lack of clear directional change in the vegetation community between years is indicated by segments oriented in many different directions.

Though community change trajectories were not apparent (Figure 3.4.), treatment did alter species composition and abundance (PERMANOVA, Treatment: $F_7=2.11$, p<0.01). Treatment effects were similar across sites (Site:Treatment: $F_{21}=1.07$, p=0.21), but individual species responses to treatments were generally not consistent. Exceptions included two abundant erect shrubs at the DAW site where bog bilberry (*Vaccinium uliginosum*) and Labrador tea (*Rhododendron groenlandicum*) both increased in cover substantially in the triclopyr point injection plots. In addition, the dominant bunchgrass at the CAR, HJ1 and HJ2 sites, purple

reedgrass, decreased in cover in all plots treated with imazapyr. Changes in cover of dominant species between 2014 and 2015 are summarized in Appendix 2.

Species richness and evenness were homogeneous among plots within each site prior to treatment (richness: p=0.79, evenness: p=0.69) and different among sites (p<0.01). One year after treatment, species richness and evenness remained different between sites, but only species richness was affected by treatment (Figure 3.5.). Mean species richness was lowest in imazapyr broadcast spray plots, highest in imazapyr point injection plots. There was no interaction between site and treatment.



Figure 3.5. Average and by site species richness and evenness one year after eight ROW management treatments in Yukon (\blacksquare = Average, \bullet = CAR, \blacktriangle = DAW, \circ = HJ1, \square = HJ2). Richness and evenness differed by site (Richness: F_{3,62}=23.1, p<0.01 and Evenness: F_{3,62}=17.29, p<0.01), however treatment only influenced species richness (Richness: F_{7,62}=3.44, p<0.01, Evenness: F_{7,62}=1.82, p=0.10). Interactions between site and treatment were not significant for either (Richness: F_{21,62}=1.04, p=0.43 and Evenness: F_{21,62}=0.98, p=0.50). Different letters indicate statistically significant differences between least square means. Treatment codes are described in Table 3.4.

3.5 Discussion

Treatments successfully altered vascular plant communities within one year. Control of target species one year following treatment ranged from 66-94% in chemically treated plots while mechanical removal of targets with or without grass seeding provided less than 55% control. Disturbance of non-target species due to treatments generally caused a neutral or minor negative change in erect shrub, prostrate shrub, forb and graminoid cover. Both target and non-target vegetation displayed signs of imazapyr damage one year after application. Leaf chlorosis, stunted growth and tissue deformity of many species occurred after broadcast spraying, as well as, selective cut stump and point injection treatments. Herbicide damage to non-target species that were not directly treated suggests imazapyr transferred from target species by an unknown belowground mechanism. Other treatment effects were life form or species specific which is encouraging; treatments were designed to impact life forms differently based on species' height and physiology (monocot vs. dicot) to induce different recovery trajectories. Additional species-specific changes in cover are listed in Appendix 2.

3.5.1 Control of Target Species

Control of target species by chemical treatments one year following application was highly successful. Our study demonstrates that triclopyr and imazapyr applications on northern ROWs are just as effective at short-term woody species control as they are in southern jurisdictions (Bramble et al. 1991; Luken et al. 1991; Mercier et al. 2001; Yahner and Hutnik 2004). Chemical treatments reduced target species cover by as much as 94% of their original abundance compared to less than 50% reduction by mowing. The high level of control also confirms the findings of Seefeldt et al. (2013), who demonstrated that 2,4-D and triclopyr can be effective for woody species control in northern conditions. Recovery of target species in chemical treatment plots to mowing control levels is unlikely to occur rapidly as damage assessments of treated stems indicated nearly lethal damage.

Poplar stems were significantly less visually damaged than birch and aspen, though this was not evident in the percent control analysis. Differences in herbicide sensitivity between aspen and poplar have also been reported elsewhere, however which species is more susceptible depends on the herbicide's active ingredient (Sharma and Vanden Born 1970; Bowes and Spurr 1996). Whether less visual damage indicates poplar will recover faster than other target species will require future measuring.

3.5.2 Response of Non-Target Vegetation

A small decrease in total non-target vascular plant cover after one year was observed across all eight treatments. This is not unexpected and decreases in shrub and herbaceous cover are commonly reported one year after both mechanical and chemical site preparation techniques in boreal clear cuts (Sullivan et al. 1996; Man et al. 2010). As treatments were applied by hand, trampling damage would also have occurred in all plots. The recovery of forbs and substantial increase in grass cover two years after triclopyr broadcast spray and cut stump applications has been demonstrated in both boreal forests and rangelands (Bell and Newmaster 2002; Seefeldt et al. 2013). It is likely that herbaceous species abundance on Yukon ROWs will increase by the second year after triclopyr treatments. Recovery time for shrubs after triclopyr applications is less consistent ranging from 2-5 years (Bell and Newmaster 2002; Seefeldt et al. 2013). In contrast, imazapyr treatments continued to cause visible herbicide damage to non-target vegetation after one year and may inhibit species recovery for a prolonged period.

Imazapyr broadcast spray, though most effective at controlling target species, also caused greater visual damage and cover reduction to non-target vegetation than other treatments. Prostrate shrubs were the exception and the most common species, kinnikinnick, appeared to tolerate imazapyr well. In addition to damage after spraying, imazapyr damage to non-target species that were not directly treated also occurred in cut stump and point injection plots. Chlorosis, deformity and stunting of forbs was common after both treatments, but severity was highly species specific. The variability in sensitivity confirms plant species exhibit a large range of tolerances to imazapyr (Bovey and Senseman 1998; Douglass et al. 2016). Graminoids were not as visually damaged as forbs, although graminoid cover declined significantly after cut stump and to a lesser degree, point injection treatments. Erect shrubs had visible imazapyr damage in point injection plots and this was also reflected in a reduction of cover. Species richness was not reduced after imazapyr cut stump or point injection treatments but was lower in broadcast spray plots, indicating non-target plants were exposed to smaller imazapyr concentrations from selective treatments than from broadcast spray. These imazapyr concentrations in cut stump and point injection plots were not toxic enough to prevent non-target species germination, but had sufficient potency to cause visible tissue deformation and damage.

The substantial damage to and in some cases cover reduction of non-target species with selective application of imazapyr suggests some form of belowground transfer of imazapyr from

treated stems. Non-target impacts from selective imazapyr treatments have been noted previously (Kochenderfer et al. 2001; DiTomaso and Kyser 2007; Lewis and McCarthy 2008). Potential transfer mechanisms include indirect soil contamination by root exudation and/or leaf senescence or direct transmission through mycorrhizal fungi or root grafts (Lewis and McCarthy 2008). Root exudation of imazapyr has been demonstrated in both woody and herbaceous plants and, once excreted, imazapyr is relatively mobile in soil and can be reabsorbed by other plant roots (Kanampiu et al. 2002; Silva et al. 2004). Imazapyr translocation studies also indicate that much of foliar applied imazapyr remains in the treated leaves (Tucker et al. 1994; Bernards et al. 2009) and contamination of soil by herbicide residues from decomposing plant material has been observed (Newton et al. 1990; Ranft et al. 2010). Nutrient transfer between plants through mycorrhizal networks is well documented (e.g. Simard and Durall 2004) as is the transfer of allelochemicals (Barto et al. 2011, Achatz et al. 2014). Imazamox, an imidazolinone herbicide closely related to imazapyr, was used an experimental surrogate for an allelochemical and was transferred between a treated and untreated plant exclusively through mycorrhizal connections (Barto et al. 2011). Mycorrhizae are abundant in boreal soils (Lindahl et al. 2007) and these networks between vascular plants likely exist on Yukon ROWs. There is little known about herbicide transfer through root grafts, however the transfer of 2,4,5-T in sweetgum (Liquidambar styraciflua) has been reported (Fenton 1965). Regardless of the transfer mechanism, herbicide damage assessments clearly indicated continual imazapyr activity 365 days after both selective and non-selective applications.

Other than imazapyr causing more visible damage to non-target species, responses to treatments were life form or species specific. These results are encouraging as treatments were designed to selectively disturb species based on their morphological and physiological characteristics. For example, cut stump herbicide applications targeted individual plants of 20 cm height or greater with the intent of not disturbing low growing vegetation while broadcast spray applications favour species with intrinsic tolerance of the herbicide active ingredients. Cut stump and broadcast spray treatments would therefore promote different species and thus encourage the development of different plant communities. The two cover types we identified as having the most potential for inhibiting target species establishment on Yukon ROWs are shrub or grass dominated communities. Neither the use of a broadleaf selective herbicide (triclopyr) nor the additional seeding of a native grass seed mix increased graminoid cover after one year, though new seedlings

were noted at all sites. The point injection and selective cutting methods intended to promote erect shrub cover were only partially successful after one year as selective cutting and point injection of triclopyr resulted in a neutral or positive cover change. The most substantial increase in erect shrub cover was at the DAW site where bog bilberry cover increased by >15% in triclopyr point injection plots. Cover of bog bilberry did not increase when only aboveground target species biomass was cut and removed in selective cutting plots. Triclopyr point injection treatments provided substantial control of target species and a release from competition could explain the increase in bog bilberry cover. Though these results are very preliminary, they are the first indications of suitable shrub species for moist, acidic sites.

Understanding species-treatment interactions is critical to selecting effective management methods that promote desirable cover types. The dramatic decrease in prostrate shrub cover one year after triclopyr broadcast spray applications is a good example of why knowledge of specific herbicide-species impacts are important. Kinnikinnick accounted for 68% of the total prostrate shrub cover across sites prior to treatment and damage assessments confirmed the species was highly sensitive to foliar applied triclopyr. Triclopyr broadcast spray is thus a less desirable choice for a site like HJ1 where kinnikinnick comprises a large percentage of the understory cover and its low growth form is compatible with ROW management objectives. Further monitoring measurements are required to confirm additional relationships between treatments and species' abundances as plant communities continue to respond after vegetation management disturbances.

3.5.3 Vascular Plant Community Change

Herbicide applications to boreal ecosystems can cause both short and long term effects on plant communities (Strong and Sidhu 2005). Significant changes to the vascular plant communities in response to treatments occurred at our sites after one year as species responded to the disturbances differently. Clear directional changes of plant communities as a whole, however, were not yet apparent. Only two treatments had an effect on species richness with lower richness in imazapyr broadcast spray plots and higher in point injection with imazapyr. All other treatments' species richness values were similar and evenness was consistent between all treatments. Long-term studies of glyphosate and hexazinone site preparation treatments show few impacts on boreal species richness and evenness (Sullivan and Sullivan 2003; Strong and Sidhu 2005), which suggests that such changes should not be expected. When a change in richness is

reported after boreal site preparation, it is often an increase associated with invasion of weedy species (Bell and Newmaster 2002); weed invasion was not observed at any of our sites.

Evidence of early community changes indicates the initial floristic compositions of treatment plots were altered and the development of new, distinct communities can be expected over time (Egler 1954; Niering 1987; Strong and Sidhu 2005). In the boreal forest, the most important changes in species composition and abundance occurs in the year following disturbance (de Grandepre and Bergeron 1997) and this likely applies to Yukon ROWs. The ability to characterize future communities and their capacity to resist the regrowth or invasion of target species, however, is limited by the length of our study. Yukon ROWs are currently mowed every 8-10 years and at least one cycle is needed to fully evaluate community development. How species-treatment relationships are influenced by inter-annual weather variations was also not explored as treatments were only applied in one season. Vegetation responses to treatments were similar among sites, suggesting weather did not significantly affect plant responses, but this relationship was not examined directly Future studies would benefit from employing larger treatment areas and use of operational equipment (i.e. use of mowers and wet blading equipment versus hand cutting) to better determining large scale operational conditions. As monitoring continues and knowledge of the ecosystem dynamics increases over time, vegetation management strategies can be adapted and improved. This continuous evaluation and adaptation of management techniques is fundamental to Integrated Vegetation Management (Nowak and Ballard 2005) and our study provides the foundation for the development of an IVM program for Yukon ROWs.

3.6 Conclusion

Understanding vegetation dynamics and how those dynamics are influenced by management methods are critical components of IVM for power line ROWs. We evaluated eight management treatments on their control of target species and impacts on non-target plant species and communities. Changes in vascular plant composition and abundance were successfully induced one year after treatments. Developments into new, distinct plant communities were not year clear due to the short timeframe of the study, however strong species-specific responses to treatments were detected. Our study demonstrates that even in cool northern conditions, effective short term control of target species can be achieved with herbicide applications of triclopyr and imazapyr. Triclopyr treatments were much more successful than imazapyr treatments at minimizing damage and cover reduction of non-target vegetation. The prostrate shrub, kinnikinnick, was an exception and significantly more susceptible to broadcast spraying of triclopyr than imazapyr. Selective application of triclopyr (i.e. cut stump and point injection) was effective at controlling target species one year following application and also reduced impact on non-target species compared with broadcast spraying.

Both selective and non-selective imazapyr treatments caused deformity and chlorosis of nontarget vegetation one year after application, though severity was species specific. This strongly suggests imazapyr can remain active in the soil for more than one season in northern conditions; longer than the typical 25-142 day half-life (Senseman 2007). Damage to species that were not directly treated also indicates that imazapyr can also transfer through an unknown belowground mechanism. The residual activity and transfer mechanism of imazapyr raises concerns about its use on Yukon ROWs. The range of sensitivities of northern boreal plants to imazapyr and triclopyr applications, as well as, the potential for their persistence in soil also highlights the need for more focused toxicity research in the North.

3.7 References

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Preamble: Chapter 4

Changes in plant community composition after imazapyr and triclopyr applications and the range of sensitivity to the herbicides demonstrated by non-target plants raise concerns about the vulnerability of non-target species. Fireweed (Chamerion angustifolium) and varrow (Achillea *millefolium*) were the most frequent and abundant forbs at all the field sites and are important sources of food for wildlife such as snowshoe hare, moose, and bees. Both species are also culturally important to Yukoners for edible or medicinal uses. Acute toxicity tests for terrestrial plants provide a standardized assessment of fireweed and yarrow sensitivity to imazapyr and triclopyr, which can be used to help interpret results from the field experiment. Seed was sourced from northern locations and the seedling emergence and seedling growth test was conducted using soil from each field site. This chapter also builds upon the growing body of work on toxicity testing with non-crop plants and includes bluejoint reedgrass (Calamagrostis canadensis) in the seedling emergence and seedling growth test. Bluejoint was recently designated as a standard test species by Environment Canada for testing for contamination of boreal soils the only standard species that was considered non-target and occurred at one of the field sites. This chapter provides a summary of each species' performance as test organisms to further the development and application of native plants for use in ecotoxicity testing.

4.0 HERBICIDE TOXICITY TESTING WITH NON-TARGET BOREAL PLANTS: SENSITIVITY OF YARROW AND FIREWEED TO TRICLOPYR AND IMAZAPYR

4.1 Abstract

Standardized terrestrial plant toxicity tests were conducted to determine the sensitivity of two boreal plants, yarrow (Achillea millefolium L.) and fireweed (Chamerion angustifolium L.), to the herbicides imazapyr and triclopyr. A. millefolium and C. angustifolium are common colonizers of disturbed boreal ecosystems, including northern powerline rights-of-way (ROWs), and the impacts of proposed herbicide applications on non-target plants are of concern. In the vegetative vigour test, triclopyr foliar spray caused extensive damage to A. millefolium at <50% of the maximum field application rate (Inhibition Concentration (IC)₅₀=1417.9 g a.i. ha⁻¹) and was completely lethal to C. angustifolium at the lowest dose (1132.5 g a.i. ha^{-1}). A. millefolium and C. angustifolium demonstrated extremely high sensitivity to imazapyr foliar spray: IC₅₀=8.6 g a.i. ha⁻¹ and 5.0 g a.i. ha⁻¹ respectively (<1.5% of the maximum field rate). A foliar application of either herbicide to control woody species would likely cause significant damage to both species. The seedling emergence and seedling growth tests were conducted in the organic horizon of five field collected northern boreal ROW soils. Few differences in herbicide uptake between soils and few differences in sensitivities between species were detected. Triclopyr limited growth of A. millefolium and C. angustifolium at relatively low levels (most IC₅₀ estimates between 2-20 µg g⁻ ¹). For imazapyr, IC₅₀ estimates could not be calculated as there was >75% inhibition of most endpoints at the lowest doses of $\sim 2 \ \mu g \ g^{-1}$. High sensitivities of non-target plants to low concentrations of imazapyr and triclopyr in soil suggests long term impacts of herbicide applications are dependent on herbicide degradation rates in northern conditions and more research in this area is needed. A. millefolium performed well as a test organism and is recommended for use in standard toxicity testing relevant to boreal regions.

4.2 Introduction

Ecotoxicity testing of pest control products is a key component of pesticide regulation. There are two tests used to characterize acute herbicide toxicity to terrestrial plants: the vegetative vigour test and the seedling emergence and seedling growth test (OECD 2006, USEPA 2012). The vegetative vigour test evaluates the sensitivity of young plants to foliar spray while the seedling emergence and seedling growth test assesses soil herbicide concentration effects on seed

germination and early seedling growth. For regulatory purposes each test is typically completed on 6-10 crop species from multiple families to encompass the range of non-target plant sensitivities. There is considerable debate as to whether non-target species sensitivity is adequately represented in these tests (McKelvey et al. 2002; Boutin et al. 2004; Clark et al. 2004; White and Boutin 2007). The range of sensitivities to herbicides can vary more between wild species than agricultural ones and even if the range is accurate, most regulatory testing does not provide species-specific information for non-crop, non-target plants (Olszyk et al. 2008). A "List of Potential Non-Crop Species" was added as an annex to the Organisation of Economic Development and Cooperation (OECD) guidelines in 2006 to encourage the representation of nontarget plants in regulatory testing (OECD 2006). In addition, Environment Canada recently released a method for assessing the phytotoxicity of potentially contaminated boreal soils with seven boreal plant species (2013). The use of non-crop plants presents challenges for testing: homogenous "crops" of wild plants for vegetative vigour tests are difficult to produce due variability in plant growth rates and morphology (Pallett et al. 2007) and genetic variability (e.g. ecotypes) also increases the range of responses within a species (Boutin et al. 2010). Many argue, however, that testing non-target plants is essential to understand potential off-target impacts of herbicide applications (White and Boutin 2007; Olszyk et al. 2008; Boutin et al. 2012).

Knowledge of non-target plant responses to herbicide applications is an important component of Integrated Vegetation Management (IVM) for power line rights-of-way (ROWs) (Nowak and Ballard 2005). Herbicides are commonly used for woody species control on seminatural ROWs in southern Canada and the United States (Sulak and Kielbaso 2000). Preserving or enhancing low-growing plant species has been positively associated with slower regrowth or invasion of incompatible trees on ROWs (Bramble and Byrnes 1983; Dreyer and Niering 1986; Mercier et al. 2001). ROW plant communities can also be managed to provide wildlife habitat or increase ecosystem biodiversity (Russell et al. 2005; Clarke et al. 2006). Identifying management methods that have minimal impact on desirable plant species involves understanding non-target plant responses to treatments (Luken et al. 1994; Nowak and Ballard 2005).

In regions where native plant species sensitivity to herbicide applications is not well documented, terrestrial plant acute toxicity tests provide a standardized method to quantify and compare sensitivities of non-target plants to herbicides. The Yukon Territory, in northern Canada, has >1000 km of power line ROWs that are managed by mechanical mowing. Chemical vegetation

management strategies are currently being explored, however local information on impacts to boreal non-target plants is minimal (Chapter 3). Shrub control research in Alaska indicates the herbicide sensitivity of boreal plants is species specific (Seefeldt et al. 2013) and two native species were selected for focused toxicity testing.

Achillea millefolium and Chamerion angustifolium are common colonizers after anthropogenic disturbance in Yukon (Lister 2009) and the dominant herbs on Yukon ROWs (Chapter 3). A. millefolium and C. angustifolium are rhizomatous perennials of ecological and cultural importance in the region. Snowshoe hare (*Lepus americanus*), a keystone boreal species, feeds on both plant species during the late summer (Seccombe-Hett and Turkington 2008). C. angustifolium is also particularly attractive to bees and other pollinators (Kevan et al. 1993) and a key component of moose summer diet (Johanson et al. 1994). Both species are also harvested as edible and/or medicinal plants (Gray 2012). If A. millefolium and C. angustifolium perform well as test organisms, their inclusion in standard toxicity tests for industrial chemicals would better represent plant communities of disturbed boreal sites than the crop species often used (Princz et al. 2012).

Two herbicides were studied as candidates for use on Yukon ROWs: triclopyr and imazapyr. Triclopyr (commercial formulation Garlon XRT, 755 g L⁻¹ triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) is a Group 4 herbicide and acts as an auxin mimic. Triclopyr has low leachability and the majority deposited on the forest floor remains in the organic layer (Lee et al. 1986; Thompson et al. 2000). Imazapyr (commercial formulation Arsenal Powerline, 240 g L⁻¹ imazapyr acid; BASF Canada Inc., Mississauga, ON) is a broad spectrum Group 2 herbicide in the imidazolinone family and inhibits the production of three amino acids by binding to the acetolactate synthase (ALS) enzyme. Imazapyr can be applied pre- or postemergence and can remain mobile in soils for an extended period of time (Loux and Reese 1993; Bovey and Senseman 1998; Gianelli et al. 2014). Both herbicides are degraded in soil through microbial breakdown and photolysis on the soil surface (Curran et al. 1992; Johnson et al. 1995; Gianelli et al. 2014). The bioavailability and persistence of triclopyr and imazapyr can differ depending on soil characteristics such as pH, organic carbon and percent clay as well as environmental conditions affecting microbial activity (Wehtje et al. 1987; Loux and Reese 1993; Johnson et al. 1995; Newton et al. 2008; Allison et al. 2013; Gianelli et al. 2014; Douglass et al. 2016).

Phtyotoxicity testing with *A. millefolium* and *C. angustifolium* in Yukon ROW soils facilitates the direct examination of imazapyr and triclopyr toxicity to two boreal non-target plants in northern soils. The objectives of this study were to 1) determine the acute toxicity of imazapyr and triclopyr as foliar spray to *A. millefolium* and *C. angustifolium*; 2) determine the acute toxicity of imazapyr and triclopyr in five field collected Yukon ROW soils and compare the results to a standard boreal test species, *Calamagrostis canadensis* and 3) evaluate *A. millefolium* and *C. angustifolium* performance as potential standardized test organisms for both the vegetative vigour and seedling emergence and seedling growth tests.

4.3 Material and Methods

4.3.1 Seed Sources

Seeds for the vegetative vigour test were donated by the Alaska Plant Materials Center (Palmer, AK). *Achillea millefolium* seed was collected from cultivated plants at the Alaska Plant Materials Center Farm and *Chamerion angustifolium* seed was gathered from a wild stand in western Alaska. The same *A. millefolium* seed was used for the seedling emergence and seedling growth test, but a second lot of wild *C. angustifolium* seed from central Yukon (62.9°N, 139.1°W) was used due to insufficient germination. *Calamagrostis canadensis* was included in the seedling emergence and seedling growth test as *C. canadensis* was the only non-target boreal species listed by Environment Canada (2013) that occurred on the ROW research sites. *C. canadensis* seed was donated by BrettYoung (Winnipeg, MB).

4.3.2 Vegetative Vigour Test

The vegetative vigour test was conducted at the University of Saskatchewan Agriculture Research Greenhouses between January and April 2015, following the OECD Test. No. 227 protocol (OECD 2006). A range finding test of four doses (0.5, 1, 2, and 10 times the maximum field application rate) was conducted prior to definitive testing to identify the approximate lowest lethal dose for each herbicide-species combination. Limited germination by *C. angustifolium* prevented testing the full range of doses. A visual damage assessment was conducted 28 days after treatment using a 0-100 scale, with 0 being no damage and 100 being dead (Appendix 9).

For the range finding and definitive tests, 10 cm x 10 cm pots were filled with moistened commercial potting soil (Sunshine Mix #4, Sun Gro Horticulture, Agawam, MA) and five seeds planted in each pot. In response to the poor emergence in the range finding test, *C. angustifolium*

seeds were planted 12 days before *A. millefolium*, watered deeply and placed in a "cold storage" room (~4°C) for cool, moist stratification. In the greenhouse, pots were placed in trays lined with capillary mats and moisture was checked daily. Trays were watered daily or every other day depending on external conditions. Greenhouse temperatures averaged 24°C and fluctuated \pm 4°C. The photoperiod was 16 hours light/8 hours dark with natural light supplemented by high pressure sodium lighting. Soluble fertilizer was applied once per week at 100 ppm 20-20-20 with micronutrients as part of the watering regime. Minor pest outbreaks were biologically controlled with predatory mites. Trays were randomized three times per week. Plants were thinned the day before spraying by pinching extras above the soil surface to allow for selection of uniformly sized individuals.

Doses for *A. millefolium* and *C. angustifolium* followed a logarithmic scale with the lowest lethal dose identified in the range finding test as the highest concentration (Table 4.1.). An error in calculation for triclopyr doses resulted in testing between 0.25 and 1 times the application rate only. Solutions of imazapyr and triclopyr for both tests were prepared from commercial formulations Garlon XRT (755 g L⁻¹ triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) and Arsenal Powerline (240 g L⁻¹ imazapyr acid; BASF Canada Inc., Mississauga, ON). Fifty to sixty mL of the highest doses in each set were mixed in the laboratory using deionized (DI) water as the solvent to attain the correct concentration of active ingredient. Subsequent doses of 30 mL were prepared using the highest dose mixture and diluting with DI water. The oil based spray adjuvant Hasten (Victorian Chemical Company Pty. Limited, Coolaroo, Australia) was added to each Arsenal dose at a rate of 0.25% by volume.

Table 4.1. Tests, species and doses used to assess acute phtyotoxicity of imazapyr and triclopyr to northern boreal plant species *A. millefolium* and *C. angustifolium*. Seedling emergence and seedling growth doses are approximate as the maximum application rate is based on area (g a.i. ha⁻¹) and required estimated conversion to volume (μ g a.i. g⁻¹) (see Equation 1.). Highest dose is the highest concentration used to calculate the logarithmic dose series. * indicates an additional higher dose not included in logarithmic dose calculation. Times Max. Rate refers to the number of times the maximum field application rate (triclopyr: 4530 g a.i. ha⁻¹; imazapyr: 720 g a.i. ha⁻¹). Select characteristics and locations of test soils (CAR, DAW, HJ1, HJ2, LS) are described in Table 4.2.

Test	Species	Herbicide	Highest Dose VV (g a.i. ha ⁻¹)	Times Max.	# of Doses
* *		T : 1	$\frac{\text{SESG} (\mu \text{g a.i. g}^{-1})}{4\pi}$	Rate	
Vegetative	A. millefolium	Triclopyr	45,300	10x	9
Vigour (VV)		Imazapyr	720	1x	9
	C. angustifolium	Triclopyr	4530	1x	8
		Imazapyr	720	1x	8
Seedling	A. millefolium	Triclopyr	CAR – 301.0	~10x	8
Emergence and			DAW – 293.0		
Seedling			HJ1 – 183.1		
Growth (SESG)			HJ2 – 136.5		
			LS – 164.5		
		Imazapyr	CAR - 600.0	~50x	8
			DAW – 584.1		
			HJ1 – 365.0		
			HJ2 - 272.0		
			LS – 328.0		
	C. angustifolium	Triclopyr	CAR – 55.8, 301.1	~2x, 10x*	8
			DAW - 54.3, 293.0		
			HJ1 – 31.6, 183.1		
			HJ2 – 22.6, 136.5		
			LS – 28.0, 164.5		
		Imazapyr	CAR – 12.0, 600.0	~1x, 50x*	8
			DAW – 11.8, 584.1		
			HJ1 – 7.3, 365.0		
			HJ2 – 5.4, 272.0		
			LS – 6.6, 328.0		
	C. canadensis	Triclopyr	CAR – 689.2	~22x	8
			DAW - 668.7		
			HJ1 – 394.0		
			HJ2 – 283.1		
			LS – 349.3		
		Imazapyr	CAR - 600.0	~50x	8
			DAW - 584.1		
			HJ1 – 365.0		
			HJ2 – 272.0		
			LS – 328.0		

A. millefolium was sprayed 22 days after planting at the 2-4 true leaf stage. *C. angustifolium* emerged and grew slightly slower and was sprayed 25 days after planting. The early development

pattern of *C. angustifolium* resulted in 6-10 true leaves at the time of spraying. A custom built track sprayer was used at the University of Saskatchewan (Agassiz Scientific Ltd. of Saskatoon, Saskatchewan). The sprayer was calibrated to 218 L ha⁻¹ at a speed of 4.5 km hr⁻¹ and pressure of 40 psi. The nozzle used was a TeeJet 8002E flat fan (Spraying Systems Co., Wheaton, IL) adjusted to 50 cm above pots. Treatments were applied in sequence starting with the lowest dose. Nozzle and container were washed with soap and water between herbicides and rinsed three times with DI water. Each plant was clipped at the soil surface 28 days after treatment and placed in a drying oven at 70°C. After 72 hours in the oven, aboveground biomass was weighed to the nearest 0.1 mg.

4.3.3 Seedling Emergence and Seedling Growth Test

Test soils were collected from five right-of-way research sites located throughout Yukon, Canada (Table 4.2.). To maintain consistency with a separate study examining invertebrate communities, only the organic horizon was collected. Three to five collection areas were identified within each site and were cleared of leaf litter and woody debris by raking. Soils were air dried at room temperature after collection and stored in a dark shed outdoors through the winter. Before use, soil was thawed and sieved to 4.75 mm.

Table 4.2. Yukon right-of-way research site locations, percent covers of <i>A. millefolium</i> and <i>C.</i>
angustifolium, and select characteristics (± standard error) of organic soil layers. Methods for th
determination of soil characteristics are listed in Appendix 7.

		Percent Cover		Bulk		Total		
Site	Coordinates	A. millefolium	C. angustifolium	Density (g/cm ³)	рН	Organic Carbon	Total Nitrogen	
CAR	61.8° N, 136.0° W, 61.9°W, 136.1°N	0.6%	7.0%	0.20 ± 0.04	6.3 ±0.12	30.1% ±1.23	1.84% ±0.018	
DAW	63.9°N, 138.4°W	1.3%	6.0%	0.21 ± 0.05	4.5 ± 0.19	$27.5\% \pm 1.81$	$1.25\% \pm 0.013$	
HJ1	60.8°N, 136.6°W	1.4%	4.4%	0.33 ± 0.08	6.1 ± 0.15	$22.1\% \pm 0.82$	1.17% ±0.010	
HJ2	60.8°N, 136.0°W	1.8%	4.6%	0.44 ± 0.10	7.0 ± 0.22	12.4% ±0.89	$0.81\% \pm 0.003$	
LS	62.1°N, 135.1°W	1.4%	1.8%	0.37 ± 0.08	5.5 ± 0.16	$20.4\% \pm 2.1$	$0.89\% \pm 0.012$	

Range finding and definitive tests were conducted in the Yukon Research Centre greenhouse in Whitehorse, Yukon. Temperatures were maintained at 24° C $\pm 4^{\circ}$ C with 16 hours of 200 µmol 6 band spectrum LED light and 8 hours of darkness. The main shutter was kept closed for the duration of the experiment to avoid environmental variation over time. Relative humidity in the greenhouse averaged 30%. No pest control was required. There was a brief power outage in during the third week of the second run that cancelled the light timer for 24 hours, however heat was maintained.

Because herbicide is applied on a per area basis, Equation 1 was used to convert g a.i. ha⁻¹ doses to approximate μ g a.i. g⁻¹ soil. The highest dose for each soil was standardized based on the same application rate (g a.i. ha⁻¹) using the assumption that imazapyr and triclopyr remain within the top three cm of the upper horizon. Soils with higher bulk densities were therefore dosed with less herbicide active ingredient per g soil than soils with lower bulk densities. Three cm is a very conservative estimate of triclopyr and imazapyr movement in soil and under field conditions triclopyr and imazapyr typically penetrate 10-15 cm (Newton et al. 2008). Three cm reflects the approximate depth of soil collected for the toxicity tests, however, and represents the potential "worst case scenario" concentrations in the organic layer.

Equation 1. (Geisel 2007)

 $X \ \mu g \ kg^{-1} = (x \ g \ a. \ i. \ herbicide \ ha^{-1}) \times (ha \ (10^6 \ cm^2)^{-1})$

× (y cm estimated herbicide penetration depth in soil)⁻¹ × (1 × 10⁶ µg g⁻¹)

× (z kg (cm³)⁻¹ soil bulk density)⁻¹

A range finding test of three doses (~0.5, 1 and 10 times the maximum application rate) was completed to determine optimal range of doses for each soil-herbicide-species combination. Stock solutions of 10 mg active ingredient mL⁻¹ were mixed from the commercial formulations Garlon XRT (755 g L⁻¹ triclopyr butoxyethyl ester; Dow AgroSciences Canada Inc, Calgary, AB) and Arsenal Powerline (240 g L⁻¹ imazapyr acid; BASF Canada Inc., Mississauga, ON) in DI water. Each soil-dose combination was prepared in a tinfoil roasting pan and soil was weighed into the pan to the nearest 0.5 g. Beginning with the lowest dose, total water for the dose and corresponding stock solution amount were mixed in a glass jar before pouring into the tin. The soil was stirred until a homogenous texture was attained and covered with plastic wrap. Tins were kept covered overnight to allow for equal diffusion of herbicide. Soil trays were hand mixed once more prior to filling cups to the predetermined wet weight of for each soil. Five seeds were planted in 500 mL clear plastic containers with sealing lids (Environment Canada 2013). Triclopyr and imazapyr doses preparation and planting occurred on consecutive days to avoid cross contamination. Germination and visual damage assessments using a 0-100 scale were recorded 23 days after planting for the range finding test.

For the definitive test, doses for each soil were calculated along a logarithmic scale based off the highest lethal dose determined in the range finding test. The test was split into three runs beginning Oct. 2-3 (2 reps of *A. millefolium* and *C. canadensis*), Nov. 5-6 (3 reps of *A. millefolium* and *C. canadensis*) and Mar. 8-9 (5 reps of *C. angustifolium*). Dosed soils were prepared following methods used for the range finding test. Containers were changed for the definitive test as *A. millefolium* did not tolerate the humid conditions of the sealed clear plastic containers. Instead, a two cup system consisting of 355 mL Styrofoam cups with a hole punched in the inner cup to allow for drainage were used. Cups were filled to the predetermined wet weight for each site. Five seeds were planted per pot, with each seed gently pressed to soil surface and misted before being placed in clear plastic trays.

Cups were kept covered with plastic wrap for 10 days to ensure adequate surface moisture for germination – individual cups were misted if surface appeared dry. Cup placement in the greenhouse was randomized three times a week. Once covers were removed, cups were watered three times a week to the original wet weight for each site. After 28 days, emergence (plant shoots \geq 3 mm) and damage were recorded. To maintain microbial communities, soils were not sterilized and any volunteer plants that emerged from the natural seedbank were also recorded. Cups were either harvested immediately or frozen until processing.

The longest plant shoot and root from each cup was measured to the nearest mm. All plants from the pot were then dried at 70°C for 72 hours before being weighed to the nearest 0.1 mg. Total biomass was divided by emergence to determine mean biomass per plant.

4.3.4 Statistical Analysis

To create dose response curves for *A. millefolium* and *C. angustifolium* response to imazapyr and triclopyr foliar spray, each species-herbicide combination was modelled using non-linear regression techniques as described in Ritz et al. (2015) (see Appendix 3 for specific R coding). A Weibull four parameter model was selected to allow for asymmetrical curves around the inflection point and account for baseline growth of individuals prior to spraying. Dry aboveground biomass was the response variable with grams of active ingredient per hectare as the fixed factor. Modelling was completed in R version 3.1.2 using R library "drc" (Ritz and Streibig 2005; R Core Team 2015). When negative c parameters were generated, lower limits were constrained to baseline growth. Upper limits (d parameters) were only constrained to the mean control value in the triclopyr-A. *millefolium* model which had fewer low level doses to define the upper curve. Assumptions of normality and homogeneity of variance were assessed post-hoc by QQ plots and fitted vs. residual plots. A lack of fit test was completed in R Library "drc" function "modelFit" to compare the dose-response model to a general one-way ANOVA with a parameter at each dose level (p value >0.05 = adequate fit). Inhibition concentration estimates were generated by function "ED" in R library "drc".

In the seedling emergence and seedling growth test, the emergence of "volunteer" plants from the seedbank was noted in many pots and their effects on endpoints were graphically explored prior to modelling. Slight effects from volunteers were only seen in the HJ1 soil at high volunteer numbers and cups with ≥ 10 volunteers were removed from the dataset. Environmental differences between the two *A. millefolium/C. canadensis* runs were evaluated by one-way linear mixed models testing dose for each endpoint. Endpoints were $\log(x+1)$ transformed to meet assumptions of normality and homogeneity of variance. Standard deviations of block as a random variable were minimal and data from both runs were combined.

Emergence per pot for each species-herbicide combination was modelled separately using a Weibull three parameter model. Assumptions of normality and homogeneity of variance were assessed post-hoc by QQ plots and fitted vs. residual plots. If heterogeneity of variance was detected, a transform both sides technique was applied using boxcox optimization (Ritz et al. 2015). A lack of fit test was completed in R Library "drc" function "modelFit" (p value >0.05 = adequate fit). Effective concentrations causing 50% less emergence (EC₅₀) were generated by function "ED" in R library "drc".

Species-endpoint combinations for triclopyr tests were modelled separately using a Weibull three parameter model as triclopyr inhibited germination at high doses. Raw values were used to maintain the highest level of information and pots with no emergence were removed. Assumptions were assessed and data transformed as required following the same method applied to the percent emergence models. At high doses where only one replicate had germination, cups were removed as no variance could be calculated for the transformation. Inhibition concentration estimates were generated by function "ED" in R library "drc". IC₅₀ estimates were compared by examining the 95% confidence intervals and estimates were considered statistically different where intervals did not overlap (Princz et al. 2012).

Very high sensitivity of all endpoints to imazapyr concentrations in soil prevented the generation of dose-response curves. Emergence was not inhibited at low levels, however, and data were converted to percent of mean control and assessed visually at the lowest dose of 2 \pm 0.25 µg g⁻¹. The lowest dose was selected as 2 \pm 0.25 µg g⁻¹ was the closest concentration to the theoretical

IC₅₀ estimates. Because bulk densities differed between soils, the lowest doses ranged between $2\pm0.25 \ \mu g \ g^{-1}$. Herbicide is applied on a per area basis and soils with higher bulk densities will have less $\mu g \ g^{-1}$ of active ingredient (Equation 1.).

4.4 Results

4.4.1 Vegetative Vigour Test

Achillea millefolium and Chamerion angustifolium aboveground biomass were similarly affected by imazapyr foliar spray with IC₅₀ estimates of 0.7% (±0.3SE, n=49) and 1.2% (±0.9SE, n=30) of the maximum field application rate (Figure 4.1.). Due to a calculation error of triclopyr concentrations, a range of only 25-100% of the maximum field application rate was tested. Triclopyr spray was less acutely phytotoxic than imazapyr to *A. millefolium* with an IC₅₀ estimate of 31.3% (±22.4SE, n=55) of the maximum field application rate. The IC₅₀ estimate for triclopyr foliar spray on *C. angustifolium* was <25% of the maximum field application rate; all doses tested were lethal indicating the IC₅₀ is less than the lowest dose.



Figure 4.1. *A. millefolium* and *C. angustifolium* dose response curves for the 28 day vegetative vigour test: imazapyr or triclopyr applied as a foliar spray. 100% of maximum dose is equivalent to the maximum field application rate for woody species control (720 g imazapyr ha⁻¹, 4530 g triclopyr ha⁻¹). The IC₅₀ estimate for *C. angustifolium* and imazapyr was 1.2% of the maximum field application rate (± 0.9 SE, n=30). A dose response curve could not be generated for *C. angustifolium* response to triclopyr as all doses were lethal. IC₅₀ estimates were 0.7% (± 0.3 SE, n=49) for *A. millefolium* and imazapyr and 31.3% (± 22.4 SE, n=55) for *A. millefolium* and triclopyr. Lack-of-fit test comparing dose response curves to a one-way anova indicated adequate fit in all three models (p>0.05).

4.4.2 Seedling Emergence and Seedling Growth Test

A. millefolium had the highest emergence rate in control soils (52-80%) (Table 4.3.). *C. canadensis* emergence varied considerably by soil and *C. angustifolium* demonstrated poor emergence. *A. millefolium* emergence was more sensitive to triclopyr than *C. canadensis* emergence (EC₅₀=13.7 µg g⁻¹ ±2.40SE, n=246 vs. EC₅₀=126.69 µg g⁻¹ ±35.81SE, n=248), and *C. angustifolium* emergence was extremely sensitive (EC₅₀=1.59 µg g⁻¹ ±0.74SE, n=249). As doses increased, all species' emergence was reduced (Figure 4.2.). Effects of imazapyr were species-
specific with an *A. millefolium* emergence EC₅₀ estimate of 63.60 μ g g⁻¹ (±25.37SE, n=252) and *C. canadensis* emergence relatively unaffected at >100 μ g g⁻¹. *C. angustifolium* emergence was very erratic, but tended to decrease with increasing imazapyr concentrations between 1-10 μ g g⁻¹.

Table 4.3. Summary of *Achillea millefolium*, *Calamagrostis canadensis* and *Chamerion angustifolium* performances in the control (no herbicide) pots after the 28 day seedling emergence and seedling growth test. Endpoint means are listed with standard error and sample size. Site soil characteristics and locations are described in Table 4.2. Mean number of volunteers refers to the number of plants that emerged from the natural seedbank.

Site	Species	Mean Emergence	Mean Shoot Length (mm)	Mean Root Length (mm)	Mean Total Plant Biomass (mg)	Mean # of Volun- teers
CAR	A. millefolium	66%	27.9 ±2.2, n=10	172.1 ±18.6, n=10	4.0 ±0.5, n=10	1
	C. canadensis	60%	73.9 ± 4.9 , n=10	125.1 ±13.9, n=10	3.2 ±0.6, n=10	1.6
	C. angustifolium	36%	20.1 ±2.4, n=7	97.0 ± 20.8 , n=7	6.6 ±2.4, n=7	1.2
DAW	A. millefolium	60%	42.6 ± 5.0 , n=10	175.3 ±25.5, n=10	9.2 ±2.1, n=10	0.1
	C. canadensis	40%	97.1±19.8, n=10	83.5 ± 15.4 , n=10	5.7 ±1.9, n=10	0.1
	C. angustifolium	48%	30.2 ±5.3, n=9	65.9 ± 19.7 , n=8	7.3 ±2.2, n=10	0.1
HJ1	A. millefolium	60%	22.0 ± 2.9 , n=10	170.2 ±29.5, n=10	4.2 ±0.88, n=10	6.6
	C. canadensis	58%	69.9 \pm 6.2, n=10	149.4 ±9.2, n=10	3.8 ±0.6, n=10	5.6
	C. angustifolium	31%	31.0 ±10.8, n=9	73.4 ±20.3, n=9	14.6 ±5.2, n=9	2.7
HJ2	A. millefolium	52%	44.2 ±5.3, n=10	134.1 ±22.1, n=10	9.8 ±1.7, n=10	2.3
	C. canadensis	48%	64.2 ± 14.5 , n=9	83.5 ±18.9, n=10	4.9 ±1.4, n=9	1.7
	C. angustifolium	46%	66.3 ±5.0, n=9	210.0 ±25.2, n=9	92.1 ±18.5, n=9	3.4
LS	A. millefolium	80%	10.4 ±0.9, n=10	95.4 ±13.7, n=10	1.2 ±0.1, n=10	0.5
	C. canadensis	58%	40.8 ±4.1, n=10	114.2 ±8.1, n=10	1.0 ±0.1, n=10	0
	C. angustifolium	54%	7.8 ±1.2, n=8	19.0 ±4.2, n=8	1.0 ±0.3, n=8	0.6



Figure 4.2. Mean number of emerged plants per pot (out of 5) as a function of imazapyr or triclopyr concentrations in soil. Symbols represent mean emergence in each of the five soils tested: $\circ = CAR$, $\Delta = DAW$, + = HJ1, $\times = HJ2$, $\diamond = LS$. The top row indicates responses of each species (*Achillea millefolium*, *Calamagrostis canadensis* and *Chamerion angustifolium*) to triclopyr and the bottom row indicates responses to imazapyr. Missing regression lines indicate dose-response relationship could not be modelled.

Thirty-five IC₅₀ estimates for plant responses to triclopyr concentrations in soil were generated with a median of 5.13 μ g g⁻¹ and the majority (31/35) less than 20 μ g g⁻¹ (~25% of the maximum application rate) (Figure 4.3.). Model parameters and IC₁₀, IC₂₅ and IC₅₀ estimates are summarized in Appendix 4 and dose-response curve figures are presented in Appendix 5. IC_x estimates calculated by following Environment Canada statistical analysis protocols are listed in Appendix 6.

 IC_{50} estimates for *C. angustifolium* growth inhibition could not be modelled for the DAW and HJ1 sites due to poor emergence and extremely variable biomass endpoints across sites and doses. The comparison of confidence intervals indicated similarity of responses between most sitespecies-endpoint combinations with some exceptions. Between sites, *C. canadensis* shoot length was more inhibited in DAW soil (3.76 μ g g⁻¹ ±1.45SE, n=35) than LS soil (29.97 μ g g⁻¹ ±10.54SE, n=41). *A. millefolium* root length was significantly more inhibited in CAR soil (4.07 μ g g⁻¹ ±0.73SE, n=40) than HJ2 soil (8.07 μ g g⁻¹ ±0.97SE, n=31). IC₅₀ estimates for biomass did not statistically differ between the sites, however estimates for *A. millefolium* and *C. canadensis* in LS were considerably higher than in other soils. There were also few differences between species' responses within sites with no statistical differences in test species' IC₅₀ estimates for biomass or shoot length inhibition. *C. angustifolium* root length in HJ2 soil was one of the most sensitive endpoints (IC₅₀ = 2.50 μ g g⁻¹ ±0.35SE, n=24) and significantly differed from *A. millefolium* (IC₅₀ = 8.07 μ g g⁻¹ ±0.97SE, n=31) and *C. canadensis* (7.01 μ g g⁻¹ ±1.73SE, n=45) in the same soil.



Figure 4.3. Summary of IC₅₀ estimates from seedling emergence and seedling growth for *Achillea millefolium* and *Calamagrostis canadensis* response to triclopyr concentrations in five soils from Yukon power line rights-of-way. *Chamerion angustifolium* IC₅₀ estimates for root and shoot length are also included for the three soils with adequate germination for modelling, however emergence was still poor and estimates should be interpreted with caution. Symbols represent species ($\Box = A$. *millefolium*, $\bullet = C$. *canadensis*, and $\Delta = C$. *angustifolium*) and error bars indicate 95% confidence intervals of the mean. Site soil characteristics and locations are described in Table 4.2.

Shoot length was the least sensitive endpoint at the lowest dose of imazapyr (2 \pm 0.25 µg g⁻¹ depending on soil bulk density) across all soils (Figure 4.4.). Shoot lengths ranged between 13-

50% of the mean control shoot lengths with the exception of *A. millefolium* in LS soil (shoot length of 75.0% ±12.54, n=5). Root length and mean plant biomass were similarly sensitive with most measurements ranging from 2-23% of the mean control at 2±0.25 µg g⁻¹ imazapyr in soil. *C. canadensis* root length in HJ2 soil (root length of 25.3% ±12.64, n=4), *C. angustifolium* root length in LS soil (39.5% ±16.36, n=4), and each species' biomass in LS soil were the only exceptions (mean biomass of *A. millefolium*: 75.2% ±34.59, n=5; *C. canadensis*: 33.75% ±11.09, n=4; and *C. angustifolium*: 51.3% ±34.25, n=4). There were no observed differences in imazapyr phytotoxicity between CAR, DAW, HJ1 and HJ2 site soils. Inhibition of growth tended to be less in LS soils, but the variability in growth of all species was substantial both in control pots and dosed soils. Consistent differences in species sensitivity to imazapyr were not observed.



Figure 4.4. Seedling emergence and seedling growth test growth inhibition summary for *Achillea* millefolium, Calamagrostis canadensis and Chamerion angustifolium response to $2\pm0.25 \ \mu g \ g^{-1}$ imazapyr in five soils from Yukon power line rights-of-way. Mean response of replicates was converted to percent of mean control and error bars represent standard error (n=5,4,3,5,4,1,5,5,3,5,4,5,4,4). No error bars indicates emergence in only one replicate and standard error could not be calculated. The dashed line represents 50% inhibition of growth (IC₅₀). Symbols represent species ($\Box = A$. millefolium, $\bullet = C$. canadensis, and $\Delta = C$. angustifolium). Site soils and locations are described in Table 4.2.

4.5 Discussion

The ecotoxicity tests revealed differences in northern boreal species sensitivity based on the product and mode of entry (foliar vs. soil), but few differences were found between soils. As foliar sprays, imazapyr and triclopyr caused significant damage to *Chamerion angustifolium*. Achillea millefolium was also very sensitive to imazapyr, but showed signs of recovery after damage by triclopyr. When exposed to >10 µg g⁻¹ of triclopyr in soils, germination percentages of all species were reduced, however, *C. angustifolium* seeds were extremely sensitive. Higher concentrations relative to the application rate of imazapyr (5-12 µg g⁻¹ \approx 1x the maximum application rate) were required to inhibit germination of *A. millefolium* (EC₅₀=63.60 µg g⁻¹) and *C. angustifolium* seeds were again more sensitive. In soils, imazapyr was more phytotoxic to seedlings than triclopyr with *A. millefolium*, *C. angustifolium* and *C. canadensis* seedlings being similarly sensitive to each herbicide. Where information was available, IC₅₀ estimates of boreal species were typically within the range of standard test species, however, most endpoints were at the more sensitive end of the spectrum

From a vegetation management perspective, any broadcast spray application of imazapyr or triclopyr at rates appropriate for woody species control will likely cause significant damage to *C. angustifolium* and *A. millefolium*. The very high sensitivity to imazapyr also indicates both species could also be substantially damaged by drift from spray applications. In soil, triclopyr inhibited germination at lower concentrations than imazapyr, but imazapyr was significantly more phtyotoxic to seedlings. Even if triclopyr degrades rapidly, residues could potentially limit *C. angustifolium* germination for a short period of time after application. If imazapyr persists in soil, which is common in soils with low pH and high organic matter, the herbicide could cause significant damage to *C. angustifolium* and *A. millefolium* seedlings. If preservation of herbaceous non-target species is a management objective, use of imazapyr for woody species control on northern ROWs is not recommended.

4.5.1 Vegetative Vigour Test

The IC₅₀ estimates for *A. millefolium* and *C. angustifolium* in response to imazapyr and triclopyr foliar spray were well below 50% of the maximum application rates with *C. angustifolium* being more sensitive. There are no 28 day vegetative vigour tests with triclopyr listed on the EPA's Office of Pesticide Programs (OPP) Pesticide Ecotoxicity Database to compare our results with (USEPA OPP 2015), however above average sensitivity of *C.*

angustifolium to the foliar applications of triclopyr and a range of other herbicides is reported (Dixon et al. 2006; Seefeldt et al. 2013). *A. millefolium* and *C. angustifolium* were highly sensitive to imazapyr (IC_{50s of} 0.7% and 1.2% of the maximum application rate). High acute phytotoxicity of imazapyr to most plants was confirmed by regulatory vegetative vigour tests on seven crop species: all species had IC₂₅ values of less than 3% of the maximum field application rate (USEPA OPP 2015). This extreme sensitivity suggests negative impacts from drift could occur even if foliar spray imazapyr applications are focused on woody species.

The high phytotoxicity of both herbicides is not unexpected as the herbicides are designed for both herbaceous and woody weed control; many desirable species on northern ROWs are categorized as weeds in other settings (e.g. *C. angustifolium* competes with conifer seedlings in the boreal forestry industry (Hangs et al. 2003)). It is very likely that any effective triclopyr or imazapyr broadcast spray application for woody species control will cause significant damage to *A. millefolium* and *C. angustifolium*. The extent of damage is more difficult to predict from 28 day vegetative vigour tests. Longer term greenhouse testing (>28 days) indicates IC estimates can increase over time as some species recover (Carpenter and Boutin 2010; Brain and Hoberg 2016). In our experiment, *A. millefolium* leaves became deformed and curled after triclopyr spray applications, however subsequent development of new leaves with no obvious herbicide damage was observed in many pots 21-28 days after application. At the Yukon ROW research sites, percent cover of *A. millefolium* did not decrease significantly in triclopyr broadcast spray plots one year following treatment suggesting recovery of mature plants is possible (Chapter 3). No recovery by *C. angustifolium* after triclopyr spray or by either species after imazapyr spray was observed.

4.5.2 Seedling Emergence and Seedling Growth Test

Similar to broadcast spray applications, relatively low soil concentrations of imazapyr and triclopyr had adverse effects on *A. millefolium* and *C. angustifolium*, as well as, *C. canadensis*. Increasing concentrations of triclopyr inhibited emergence of all three species, but *C. angustifolium* emergence was much more sensitive ($EC_{50}=1.57 \ \mu g \ g^{-1}$). Regardless of endpoint, most IC₅₀ estimates for for plants in triclopyr contaminated soils were between 2-20 $\mu g \ g^{-1}$. If triclopyr remained in the upper three cm of soil (worst case scenario), 20 $\mu g \ g^{-1}$ is approximately equivalent to 25% of the maximum application rate in soils with low bulk densities (CAR and DAW). Though the majority of triclopyr residues remains in the litter and organic horizons

(Thompson et al. 2000), a portion would likely penetrate 10-15 or more cm (Newton et al. 2008) and the equivalent application rate likely would be less.

Imazapyr did not inhibit emergence until very high concentrations, if at all, but was more acutely phytotoxic to seedlings than triclopyr. Even the lowest doses of $2\pm0.25 \ \mu g \ g^{-1}$, imazapyr inhibited growth by more than 50% for most site-species-endpoint combinations. Based on the same assumptions used to calculate the maximum application rate for triclopyr, 2 $\mu g \ g^{-1}$ is approximately equivalent to 15% of the maximum imazapyr application rate for the DAW and CAR soils. Adjusting the timing of imazapyr applications is unlikely to minimize negative effects on non-target seedlings as post-emergence applications are required for woody species control. Conversion factors from $\mu g \ g^{-1}$ to kg ha⁻¹ were not reported in OPP Pesticide Ecotoxicity Database for imazapyr soil emergence testing, however 4/10 species' EC₂₅ values were below 1% of the maximum application rate of 720 g ha⁻¹ (USAEPA OPP 2015) and confirm high imazapyr phytotoxicity in soil as seen in our tests.

Plant sensitivity to imazapyr and triclopyr did not vary greatly between four of the five soils (CAR, DAW, HJ1 and HJ2). In the LS soil, most species-endpoint combinations were less sensitive. There was no correlation between the soil characteristics measured and the decreased sensitivity of plants in LS soil, however growth of control plants in uncontaminated LS soil was limited. Reduced efficacy of herbicides, including imazapyr and triclopyr, has been linked to poor plant vigour (Radosevich and Bayer 1979; Schoenhals et al. 1990; Bollig et al. 1995). The higher IC estimates in LS soil are likely the result of a soil condition limiting plant growth rather than conditions limiting herbicide mobility and bioavailability. Differences in soil characteristics, however, may have greater influence on triclopyr and imazapyr persistence in soils over time. If herbicide molecules sorb to soil particles, the herbicide is less available to soil microbes and protected from photolytic radiation which subsequently reduces degradation rates (Stephenson and Solomon 2007). Triclopyr and imazapyr are weak acids and exist mostly in their anionic state in all but the most acidic soils (Johnson et al. 1995; Pusino et al. 1997). With a negative charge, these chemicals do not typically sorb strongly to soil particles, however, iron or aluminum oxides with a positive charge can increase sorption and persistence in soil (Gianelli et al. 2014).

Predicting dissipation rates of triclopyr and imazapyr in soils is difficult as dissipation is a complex function of soil temperature, moisture, pH, organic matter and texture (Loux and Reese 1993; Pusino et al. 1994; Johnson et al. 1995; Pusino et al. 1997 Bovey and Senseman 1998;

Berisford et al. 2006; Gianelli et al. 2014). Triclopyr generally degrades rapidly, even in northern conditions. When applied to two sites in Alaska, only 1 μ g g⁻¹ or less of triclopyr ester was found in soils after 47-48 days (Newton et al. 2008). Another Alaskan field study also reported rapid degradation within 35 days after application with an estimated half-life of 10 days in soil (Ranft et al. 2010). Based on the Alaskan studies and a preliminary DT₅₀ estimate of 1 day in LS soil from the Yukon ROWs field study (A. Jimmo, pers. comm.), it is likely triclopyr will not remain persistent in soil at ecologically relevant levels beyond one growing season. Emergence inhibition of *A. millefolium* and *C. angustifolium* seeds at relatively low levels suggests there is potential for triclopyr broadcast spray to damage seeds and seedlings immediately after application, but this effect will likely be temporary.

In contrast to triclopyr's relatively rapid dissipation, imazapyr residues frequently persist into the following growing season or longer (Schoenhals et al. 1990; Coffman et al. 1993; Bovey and Senseman 1998; Alister and Kogan 2005). In Alaska, persistence of imazapyr in soil one year after application occurred at one of two sites, but was no longer detectable after two years (Newton et al. 2008). Visible herbicide damage to herbaceous and woody species 365 days after imazapyr broadcast spray applications on Yukon ROW sites also indicate imazapyr can remain active into the second growing season (Chapter 3). The combination of high sensitivities of *A. millefolium* and *C. angustifolium* to imazapyr and high potential for persistence in northern soils strongly suggests imazapyr applications could reduce many herbaceous species' abundances on disturbed northern sites such as ROWs.

4.5.3 Evaluation of A. millefolium and C. angustifolium as test organisms

Acute phytotoxicity testing with native plant species provided complimentary information for ongoing Yukon ROW research, but consistent with the observations of Pallett et al. (2007), using native plants presented additional challenges. Species performances as test organisms were variable and some required adaptations to protocols.

A. millefolium was a candidate for the non-crop species list for both terrestrial plant toxicity tests (OECD 2003a, OECD 2003b), however was not included in the final guidelines (OECD 2006). In our tests, *A. millefolium* performed satisfactorily in all five soils. Seeds exhibited no dormancy, were relatively large and easy-to-plant, and germinated well after more than a year of storage. Once emerged, *A. millefolium* accumulated biomass quickly and roots were durable when extracting plants from soil. *A. millefolium*'s major drawback was the intolerance of high humidity

in enclosed containers (Environment Canada 2013). In open pots, care also had to be taken to avoid directly watering leaves. Considering the widespread distribution of *A. millefolium* across the northern hemisphere, the herb may be a suitable candidate for inclusion in acute toxicity testing protocols for terrestrial plants.

In contrast, *C. angustifolium* was not well suited to test conditions. Seed germination requirements are variable for *C. angustifolium* (Myerscough 1980): cold stratification for 12 days improved germination considerably for the seed from Alaska, but seed from central Yukon was non-dormant. Seeds were also very challenging to plant accurately due to the small size. In the seedling emergence and seedling growth test, emergence rates from organic soils were much lower than 14 day germination tests in petri dishes. The life history of *C. angustifolium* as a pioneer species following fire events and previous research indicates *C. angustifolium* germinates more successfully on bare mineral soil than humus (Broderick 1990). Therefore, germination of *C. angustifolium* may have been significantly improved on mineral soils. Though accumulation of biomass was rapid after emergence in the vegetative vigour test, individual plant sizes were more variable than *A. millefolium*. As noted previously by Environment Canada (2013), *C. angustifolium* roots were very fragile making extraction from soil without damage very difficult in the seedling emergence and seedling growth test. We would not recommend using *C. angustifolium* as a test organism unless the species is the focus of the hypothesis and performance under test conditions is confirmed prior to testing.

C. canadensis was included in the seedling emergence and seedling growth test as the species had recently been designated as a standard test species in a biological test method for contamination of boreal forest soils (Environment Canada 2013). We met the minimum requirements for *C. canadensis* shoot length and root length in control pots, but achieved $\geq 60\%$ emergence in only one of five soils. Failure to meet validation criteria for *C. canadensis* also occurred during inter-laboratory validation of the Environment Canada test method (Environment Canada 2013) and variable performance may be attributed to seed lot, storage or varietal differences.

4.6 Conclusion

Terrestrial plant toxicity tests provided species-specific information on the sensitivity of *Achillea millefolium* and *Chamerion angustifolium* to two herbicides: triclopyr and imazapyr. The vegetative vigour test confirmed considerable damage to *A. millefolium* and *C. angustifolium* can

be expected after imazapyr or triclopyr foliar applications at any rate effective on woody species although observational evidence of *A. millefolium* recovery after triclopyr application was noted. Extremely high sensitivity of both species to imazapyr foliar spray strongly suggests non-target plants could be damaged by small amounts of drift, even if imazapyr is applied selectively to woody species. In the seedling emergence and seedling growth tests, there were few differences in sensitivities between *A. millefolium*, *C. angustifolium* and a standard test species, *Calamagrostis canadensis*. Imazapyr was more phytotoxic in soil than triclopyr and few differences in plant responses were seen between the five soils tested. The ecological consequence of herbicide concentrations in soil greatly depends on the dissipation rates of imazapyr may persist at low levels (Newton et al. 2008; Ranft et al. 2010, Chapter 3). The extreme sensitivity of all three test species to imazapyr in soil indicates significant damage to non-target plants could occur beyond the season of application.

A. millefolium and C. angustifolium were selected as test organisms for the phtyotoxicity tests as both species are widespread boreal plants common to disturbed areas and the most abundant herbs at northern ROW field research sites (Chapter 3). Though high quality seeds of row/field crop species are more readily available, the legitimacy of crop species as representatives of boreal plant communities is questionable (Princz et al. 2012). Our study demonstrated that native boreal plants can be used in standardized toxicity tests, however, confirmation of species' performance prior testing is recommended. We propose that A. millefolium be included as a test organism for terrestrial plant toxicity tests to improve representation of boreal plants in regulatory testing.

4.7 References

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5.0 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Study Synthesis

Integrated Vegetation Management (IVM) incorporates both environmental and socioeconomic values into a sophisticated system of power line right-of-way (ROW) vegetation management. An extensive range of mechanical, chemical and biological methods are now available to vegetation managers and rather than simply "resetting" succession to a previous stage, management methods can be designed to alter the plant community assemblages and abiotic conditions. These alterations can fundamentally change the direction of plant community development and potentially result in different stable states (Egler 1954; Rosenberg and Freedman 1984; Niering 1987; Pickett et al. 2009). Low-growing shrub communities or "cover types" have been proven to reduce tree establishment on ROWs and strategic management can facilitate their development (Bramble et al. 1991; Meilleur et al. 1994; Yahner and Hutnik 2004; McLoughlin 2014). ROW plant communities can also be managed to provide wildlife habitat or increase ecosystem biodiversity (Russell et al. 2005; Clarke et al. 2006). The first step in developing an IVM program is gathering information on the disturbance dynamics of local ROW plant communities (Nowak and Ballard 2005). The research presented in this thesis was designed to provide northern-specific information for power line vegetation mangers in Yukon considering adopting an IVM model. The first data chapter (Chapter 3) explored vegetation responses to potential management methods and the second data chapter (Chapter 4) investigated the phytotoxicity of herbicides imazapyr and triclopyr to non-target plants.

I evaluated the impacts of eight vegetation management methods on ROW plant communities after one year at four Yukon ROW sites (Chapter 3). The sites were located in three biogeoclimatic zones and although plant communities differed at each site, treatment effects were consistent among sites. Plant communities were altered by management methods one year after treatment. Dramatic changes in boreal plant communities occur within one to two years after disturbance and these early changes were expected (de Grandpre and Bergeron 1997). Chemical management with the herbicides triclopyr and imazapyr was more effective at controlling target woody species than mechanical cutting. Non-target species cover was reduced after all treatments and imazapyr applications caused more damage to non-target species than triclopyr.

Other treatment impacts were life form (e.g. erect shrub, forb, graminoid) or species-specific. Selective impacts of treatments on life forms/species are promising as the differences indicate treatments can be designed to meet management objectives (Niering 1987; Bramble et al. 1991). Of particular interest, the dominant and desirable prostrate shrub kinnikinnick (*Arctostaphylos uva-ursi*), was highly sensitive to triclopyr broadcast spray but tolerated imazapyr spray. Early indications of a potential shrub cover were also detected at the DAW site where bog bilberry (*Vaccinium uliginosum*) and to a lesser extent Labrador tea (*Rhododendron groenlandicum*) cover increased significantly in triclopyr point injection plots. The Initial Floristic Composition theory (Egler 1954) likely applies to northern ROWs, suggesting changes in species composition and abundance directly following disturbance are the first indications of diverging successional pathways. Repeated treatment applications may be needed to ensure complete transition to alternate, stable plant communities. Further monitoring over the duration of a management cycle (8-10 years) is required to determine whether the treated vegetation on Yukon ROWs will develop into alternate, distinct plant communities and what cover types best resist target species invasion.

Based on the literature review of power line ROW vegetation management and boreal disturbance dynamics (Chapter 2), I identified two potentially stable cover types for northern ROWs: shrub dominant or graminoid dominant communities. The increase in V. uliginosum cover after triclopyr point injection treatments at the DAW site may continue over time. Shrubby Vaccinium spp. are desirable covers on ROWs in the northeastern US (Niering and Goodwin 1974; Bramble et al. 1991) and V. uliginosum demonstrated the capacity to rapidly expand its cover (Chapter 3). To achieve a stable cover of V. uliginosum, however, repeated selective triclopyr applications will be required before V. uliginosum cover is dense enough to resist target species invasion. At the three drier sites (CAR, HJ1, HJ2), shrub cover did not increase and dense shrub covers may be unlikely where moisture is limiting. Most grasslands in the Yukon occur on dry, south facing slopes suggesting graminoid cover may be more appropriate for moisture limited sites (Vetter 2000). Though graminoid cover did not increase after either triclopyr broadcast spray (mode of action does not target monocots) or native grass seeding, I expect cover of species such as *Calamagrostis purpurascens* and *Poa glauca* to increase in these plots over time. The lack of rapid change may be due to the slow growth rates resulting from limited precipitation. As with shrub establishment, repeated treatments may be required to establish dense graminoid cover. To encourage the quicker establishment of graminoids, nitrogen fertilization may be beneficial (Matheus and Omtzigt 2011).

The efficacy of woody species control by imazapyr and triclopyr applications indicates herbicide use may be a management option for Yukon ROWs, however environmental and socioeconomic impacts must also be considered. I specifically examined the phytotoxicity and impacts of imazapyr and triclopyr applications to non-target plants.

In the field, triclopyr had fewer impacts on non-target plant species than imazapyr, with the exception of A. uva-ursi. Selective triclopyr cut stump and point injection treatments caused minimal damage to untreated species and graminoid cover was unaffected by triclopyr applications. Cover reductions of shrubs and forbs after triclopyr broadcast spray were similar to imazapyr, but the remaining vegetation did not show signs of herbicide damage after one year. Greenhouse phytotoxicity testing with Achillea millefolium and Chamerion angustifolium confirmed field application rates of triclopyr will likely cause significant damage to non-target forbs, but A. millefolium did display signs of recovery at 21-28 days after application. When exposed to triclopyr in soils, germination and growth of both species was inhibited at relatively low levels. Though acutely phyotoxic to plants was demonstrated in the greenhouse tests (Chapter 4), triclopyr does not remain active in soil for extended periods of time, even in the North (Newton et al. 2008; Ranft et al. 2010). The sensitivity of herbaceous plants can also be overestimated in greenhouse phytotoxicity testing as plants may recover over time (Carpenter and Boutin 2010; Brain and Hoberg 2016). Field research supports the short-term nature of triclopyr phytotoxicity. Decreases in boreal non-target vegetation cover occur after triclopyr applications, but forbs typically recover within two years and graminoid cover often increases (Bell and Newmaster 2002; Seefeldt et al. 2013). Shrub recovery is less consistent, but generally occurs within 5 years (Bell and Newmaster 2002). Considering herbicide damage was not evident on non-target species one year after treatment and previous research indicates recovery of non-target species within two years, triclopyr is unlikely to cause toxicological effects to plants on Yukon ROWs beyond the season of application.

In contrast, vegetation in imazapyr treated plots continued to show signs of herbicide damage one year after treatment. Chlorosis, deformity and stunting of forbs and erect shrubs were evident in imazapyr broadcast spray plots as well as in in cut stump and point injection plots where vegetation was not directly treated. The substantial damage to and in some cases cover reduction of non-target species with selective application of imazapyr suggests some form transfer if imazapyr from treated stems. Potential herbicide transfer mechanisms include indirect soil contamination by root exudation and/or leaf senescence or direct transmission through mycorrhizal fungi or root grafts (Lewis and McCarthy 2008). In the greenhouse, *A. millefolium* and *C. angustifolium* were extremely sensitive to both imazapyr foliar spray and contaminated soil. The extremely high phytotoxicity of imazapyr indicates even trace amounts as drift or trace residues in soil can cause significant amounts of damage to non-target plants. Imazapyr applications will thus favour species with intrinsic tolerance to the active ingredient (Douglass et al. 2016), as the residual activity of imazapyr documented in the field experiment is common (Coffman et al. 1993; Bovey and Senseman 1998; Alister and Kogan 2005). Imazapyr's high phytotoxicity to many non-target species, ability to transfer between treated and untreated vegetation, and persistence in soil indicates that the herbicide is not a suitable product for northern ROWs if maintaining non-target vegetation is a management priority.

Integrated Vegetation Management requires an understanding of the disturbance dynamics on power line ROWs to effectively design treatments to meet management objectives. I determined that different management techniques can alter northern boreal plant communities, chemical management methods are effective at short-term woody species control and imazapyr causes more damage to non-target vegetation than triclopyr. These results strongly support that IVM principles can be applied to northern power line ROWs. The transfer of imazapyr from treated to untreated vegetation through an unknown mechanism also highlighted the value of small scale testing prior to operational application. The developmental trajectories of boreal plant communities after treatments were not yet clear and continued monitoring of treatment plots is required determine if any low-growing stable communities establish. Once a suitable level of knowledge is achieved, management objectives beyond woody species control will need to be defined (Nowak and Ballard 2005). Socioeconomic and environmental considerations will vary between sites and treatments can then be selected to reflect local conditions.

5.2 Directions for Future Research

First and foremost, measuring vegetation changes on already established plots for three to five years would provide a better understanding of plant community development trajectories after treatments. IVM depends on the establishment of relatively stable plant communities that resist the regrowth or invasion of target species (Niering 1987). Whether treatments cause long-term changes in plant species composition and structure is therefore of primary interest to vegetation managers (Nowak and Ballard 2005). If alternate communities do establish, the dynamics of target

species regrowth or invasion will also require investigation. With increased knowledge of how treatments influence community development, larger scale trials conducted with operational equipment would be beneficial. The treatments applied in Chapter 3 were designed to mimic large scale application methods, however, factors such as edge effects could not be evaluated. In addition, impacts of large equipment such as soil disturbance and compaction were not represented in small-scale applications.

IVM is a process of treatment application, studying vegetation responses and improving techniques; "research" in the form of monitoring and adaptive management is an ongoing component. Differences in site conditions didn't impact vegetation responses to treatments within one year, however, the influence of site characteristics such as soil type or adjacent forest type will likely become more evident over time. A spatial database of environmental conditions will be needed to incorporate these variables into vegetation monitoring activities. Selection of potential treatments for each site should also include considerations beyond environmental conditions such as the social acceptability of treatments.

There is currently very limited forestry herbicide use in Yukon and public knowledge of herbicides is minimal. Historical herbicide use in the territory includes Esteron Brush Killer Herbicide (2,4-D + 2,4,5-T a.k.a Agent Orange), sprayed on the Haines-Fairbanks pipeline ROW between 1955 and 1967 (Gregor 1999). Within this context, it is not surprising that recent proposals for herbicide-based vegetation control have received very negative public feedback. For example, initial herbicide testing conducted by the local utility company in 2013 prompted considerable social backlash in the media (e.g. Ronson 2013). More recently, a permit application to use herbicides, including Arsenal Powerline (imazapyr), on the White Pass and Yukon Route railway was denied by Environment Yukon (Environment Yukon 2016). "Impacts to terrestrial habitat" including plants and animals was one of the major public concerns regarding herbicide applications and inadequate buffers to terrestrial and aquatic habitats were the deciding factor in permit rejection (Environment Yukon 2016). When scientific information that addresses public concerns regarding forestry herbicide use is available, outreach and extension activities are often the best approach to gain social license (Lautenschlager and Sullivan 2004). Though this thesis provided insight into triclopyr and imazapyr impacts on non-target plant species, information on other public concerns such as persistence of herbicides in plant tissue in northern climates is limited.

The persistence of herbicide residues in plant tissue, especially on northern ROWs, would greatly benefit from further research. The study reported in Appendix 8 indicated that triclopyr did not dissipate completely from Salix glauca foliage before leaf death (30 days). Residual levels were approximately half of initial concentrations and unlikely to degrade further in dead leaf tissue. S. glauca is a major component of moose forage and based on peak dry matter intakes of moose in summer, continuous consumption of triclopyr contaminated foliage could exceed the Reference Dose (formerly called the Acceptable Daily Intake) of 0.05 mg triclopyr/kg body weight/day (USEPA 1998; Appendix 8). This scenario is unlikely based on moose behaviour, however, the possibility of toxic effects to wildlife warrants further examination. In addition to effects on wildlife, persistence of herbicides in edible and/or medicinal plants is also of public concern. Harvesting of wild plants is a common and valued activity in Yukon. Berry and mushroom pickers were encountered at the ROW research sites during sampling indicating ROWs are actively used. Persistence of glyphosate in berries can occur in Vaccinium myrtilloides and *Rubus strigosus* when glyphosate is applied in late summer/early fall and berries are directly treated (Roy et al. 1989). Triclopyr is generally applied in early summer prior to berry development and the potential for triclopyr residues to translocate into berries as they develop is unknown. A secondary peak of residues in S. glauca 14 days after treatment suggests triclopyr can be readily translocated in a plant following soil uptake (Appendix 8). An investigation into triclopyr-berry relationships would provide a better understanding of potential health risks associated with triclopyr applications.

5.3 References

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APPENDIX 1: Species scores from NMDS ordination

Species scores from NMDS ordination based on Bray-Curtis distances of 2015 species abundance data one year after application of eight vegetation management treatments at four ROW research sites in Yukon.

NMDS1	NMDS2
0.0761	-0.3932
0.9654	-0.6968
1.0975	0.6415
-0.7067	-0.0488
-0.4141	-0.4053
0.5006	-0.4682
0.1243	-0.5469
0.1916	1.0673
-0.4373	0.5624
-0.1148	-0.1694
-0.4581	-0.8434
0.0427	-0.7364
0.6723	-1.3608
1.7024	-0.1500
1.2571	0.0983
1.2693	0.5243
-0.3298	-0.6248
-0.2187	-1.9663
-0.7398	0.5887
-0.0743	0.0965
1.3415	0.2121
-0.7405	-0.0169
-0.0467	-1.7919
-0.2406	0.1956
-0.9158	-0.9558
0.0850	-0.1913
0.1719	-0.7954
1.2523	0.0910
0.3579	1.2502
0.1916	0.4872
1.4333	0.2354
-0.2187	-1.9663
-0.1212	-0.4821
1.1810	0.0725
-0.1344	0.2806
1.2867	0.3250
	NMDS1 0.0761 0.9654 1.0975 -0.7067 -0.4141 0.5006 0.1243 0.1916 -0.4373 -0.1148 -0.4581 0.0427 0.6723 1.7024 1.2571 1.2693 -0.3298 -0.2187 -0.7398 -0.2187 -0.7398 -0.0743 1.3415 -0.7405 -0.0467 -0.2406 -0.9158 0.0850 0.1719 1.2523 0.3579 0.1916 1.4333 -0.2187 -0.1212 1.1810 -0.1344 1.2867

Species (Continued)	NMDS1	NMDS2
Eregemone capillaris	-0.3889	-0.9689
Eurybia sibirica	-0.1486	-0.2884
Festuca altaica	1.1612	0.1872
Festuca saximontana	-0.5600	-0.4990
Fragaria virginiana	-0.1380	-0.5246
Galium boreale	-0.3897	-0.4150
Gentianella spp.	-0.2280	-0.0239
Geocaulon lividum	-0.2864	0.9067
Hedysarum alpinum	-0.4210	0.0352
Hedysarum boreale	-0.1472	0.4521
Hordeum vulgare	0.2813	1.1577
Juniperus communis	0.3160	-0.8702
Juniperus horizontalis	-0.3980	-0.6236
Linnaea borealis	0.1899	-0.2380
Linum lewisii	-0.2256	-1.0606
Lupinus arcticus	1.0735	0.2089
Luzula arctica	-0.1438	0.2702
Mertensia paniculata	0.3170	-0.5024
Moehringia lateriflora	0.6337	-1.4758
Orthilia secunda	0.3732	-0.7069
Oxytropis campestris	0.1242	-0.5492
Oxytropis splendens	-0.2274	-0.2178
Parnassia sp.	0.6625	0.3084
Pedicularis labradorica	0.4835	-0.0599
Penstemon gormanii	-0.3801	-1.7229
Petasites frigidus	1.2564	0.1467
Picea glauca	-0.5524	0.1175
Picea mariana	1.3617	0.4808
Poa glauca	-0.7197	-0.5656
Poa sp.	0.6395	-1.6655
Polemonium acutiflorum	1.2393	-0.1636
Polemonium pulcherrimum	0.7179	0.1704
Polygonum viviparum	-1.1632	-0.5001
Populus balsamifera	-0.1803	-0.0531
Populus tremuloides	-0.4707	-0.1311
Pyrola spp.	0.2160	-0.5163
Ranunculus macounii	1.2264	-0.8214
Rhododendron groenlandicum	1.3846	0.3633
Rosa acicularis	-0.2225	-0.0312
Rubus arcticus	0.4569	-1.1553
Rubus idaeas	1.2317	0.1397
Salix spp.	0.6718	-0.0205

Species (Continued)	NMDS1	NMDS2
Saxifraga tricuspidata	-0.7105	0.3696
Senecio spp.	0.4811	-0.0908
Shepherdia canadensis	-0.1212	-0.1991
Solidago spp.	-0.6185	0.0841
Spiraea beauverdiana	1.7361	-0.1326
Spiranthes romanzoffiana	1.1981	-0.2971
Taraxacum ceratophorum	1.1158	-0.2290
Trifolium hybridum	1.0243	-0.2988
Trisetum spicatum	-0.3875	-0.8481
Vaccinium uliginosum	1.3907	0.3299
Vaccinium vitis-idaea	0.6135	0.5729
Viburnum edule	-0.0726	-0.6705
Zygadenus elegans	-0.8068	-0.1793
Unknown Carophyllaceae	-0.3726	-0.2988
Unknown grass	-1.0329	-0.4763
Unknown grass	-0.2914	-0.6863
Unknown grass	0.2171	0.9482
Unknown Juncus sp.	0.1305	0.5826
Unknown sedge	-0.0078	-1.3569
Unknown Viola sp.	0.9211	-0.9151
Native Grass Seed Mix	0.0154	0.2044

APPENDIX 2: Percent cover of common non-target species between 2014 and 2015

Average and by site percent cover of common non-target species changes between 2014 and 2015, one year after application of eight vegetation management treatments. Common species were defined by >5% cover in at least one plot. Treatment codes are described in Table 3.4.

Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Erect Shrubs (<1.5 m)								
Dasiphora fruticosa		-13.6			-5.4		-3.5	
Rhododendron								
groenlandicum	3.0	2.6	1.2	-8.0	6.0	-0.8	2.5	-1.8
Rosa acicularis	-3.9	-3.9	-4.0	-9.0	-2.0	-3.7	-2.7	-1.6
Rubus idaeus	-1.8	0.1	-0.6	-0.8	-1.9	0.0	-0.1	-0.1
Shepherdia canadensis	-5.4	-10.6	-0.7	-11.4	-0.3	-7.0	5.0	0.7
Spirea beauverdiana	-5.0							
Vaccinium uliginosum	-6.0	-13.2	5.9	-16.4	17.4	1.8	1.3	-1.4
Viburnum edule			-7.3		3.5	-3.5		-4.8
Prostrate Shrubs								
Arctostaphylos uva-ursi	-15.6	-2.6	3.2	2.5	-6.8	-4.0	-3.4	-6.2
Diphasiastrum								
complanatum					-1.8			4.3
Juniperus horizontalis							1.3	-5.0
Linnaea borealis	-8.7	-2.4	2.2	-1.2	-1.0	-0.8	1.8	3.5
Vaccinium vitis-idaea	-3.1	1.9	-0.8	-2.2	-2.1	-1.8	-0.9	0.0
Forbs								
Achillea millefolium	-0.5	-0.7	-0.8	0.1	0.0	-0.2	-0.5	-0.4
Anemone spp.	-1.1	-1.3	0.4	-1.2	0.2	-1.1	-0.9	0.6
Antennaria spp.	-0.5	-2.9	-3.8	-0.8	-0.3	-1.7	0.3	-0.4
Arnica angustifolia	1.5	0.1	0.8	0.6	0.2	0.3	-0.9	0.1
Astragalus spp.	-0.2	-18.8	-0.8	-1.3	-0.3	-1.1	-0.3	
Chamerion angustifolium	-2.8	-3.6	-2.3	-2.0	-2.4	-1.9	-3.7	-1.4
Cornus canadensis	-9.4	-8.1	-7.3	-17.4	3.6	0.8	-3.6	0.2
Equisetum arvense/pratense	-6.8	-1.9	-2.0	-1.1	-1.0	-0.8	-2.0	-4.5
Equisetum scirpoides	-1.6	-1.1	-0.9	0.4	0.0	0.2	-4.4	0.3
Eurybia siberica	-0.8		-0.3	-1.3	0.0	-0.9	-0.8	1.4
Fragaria virginiana	-3.7	-3.8	-0.7	-0.3	-4.9	-2.8	-1.5	-1.5
Geocaulon lividum	-1.1	-1.3		-1.3	0.0	0.0	-1.0	0.3
Hedysarum alpinum	0.3	-1.2	-0.4	-1.3	0.3	-2.5	-2.5	0.1
Hedysarum boreale	-3.8	-18.6		-1.5	-3.3	-0.8	-1.0	-8.5
Lupinus arcticus	-1.8	-0.3	0.0	-0.2	-1.5	1.2	0.0	-12.3

Average Across All Sites

Average Across All Sites								
Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Forbs (Continued)								
Mertensia paniculata	-0.4	-0.4	-2.1	-0.4	-0.1	-0.1	0.2	-1.6
Pyrola spp.		-0.1	0.3	0.3			0.3	1.1
Senecio spp.	1.1	0.3	1.4	0.1	0.4	1.6	-0.8	0.0
Solidago spp.	-1.5	-0.1	-0.7	0.3	0.1	1.5	0.0	-1.0
Taraxacum ceratophorum	-1.9	-0.9	-0.6	-0.8	0.8	1.0	-9.9	-3.0
Trifolium hybridum						-3.4		
Zygadenus elegans	-0.1	-0.5	-0.8	-0.3	1.3	-0.3	-4.0	
Graminoids								
Bromus pumpellianus	0.4	-7.7	-0.5	-0.8	0.5	-0.5	-2.2	-1.8
Calamagrostis canadensis	0.8	1.8	0.8	0.3	0.8	1.5	0.8	0.8
Calamagrostis								
purpurascens	1.3	-10.3	-1.8	-9.3	-1.9	-8.0	-2.0	-4.6
Carex spp.	-1.8	-0.9	-0.2	-0.4	0.2	-0.9	0.0	-0.5
Elymus trachycaulus	0.5	-4.3	12.5	-1.2	0.1	1.4	0.5	1.0
Festuca altaica	-0.9	2.0	4.0	-4.4	-0.1	-4.9	0.6	2.2
Festuca saximontana	-0.2	-2.1	1.1	-0.6	0.1	-1.3	-0.3	-1.3
Luzula arcticus				-3.8	-2.5			-5.0
Poa glauca	-2.8		-5.0		-0.8	-1.3		-4.5
Trisetum spicatum	0.1		0.3	0.5	0.3			
Seed Mix					2.0	1.6	1.0	

CAR								
Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Erect Shrubs (<1.5 m)								
Dasiphora fruticosa		-13.6			-5.4		-3.5	
Rosa acicularis	-9.7	-3.8	-7.6	-11.0	-4.7	-8.5	-7.8	-2.0
Shepherdia canadensis					3.0	-13.5		3.0
Viburnum edule			-7.3		3.5	-3.5		-4.8
Prostrate Shrubs								
Arctostaphylos uva-ursi	-5.2	-0.8	5.0	-3.8	-10.6		-10.0	-1.4
Linnaea borealis	-2.3	-16.8	4.8	-1.3	-2.6	-6.9	4.9	1.8
Forbs								
Achillea millefolium	0.0	-0.6	-0.1	0.1	0.0	-1.0	-0.8	0.1
Anemone spp.	-1.6	-0.4	0.4	-1.3	0.8	-0.9	-1.5	1.3
Arnica angustifolia		-0.3		1.0			-2.5	
Chamerion angustifolium	-2.8	-2.8	-0.8	-2.0	-7.3	-3.1	-5.5	1.6
Equisetum		-3.0		-0.9	-0.1		-53	
arvense/pratense		5.0		0.7	0.1		5.5	
Equisetum scirpoides	-6.3	-5.8	-3.4	-0.3	0.2		-21.3	
Eurybia siberica	-1.3		-0.3	-0.8		0.0	-3.5	
Fragaria virginiana	-0.3		-3.5			-5.1	-5.6	
Geocaulon lividum					0.1		0.5	
Hedysarum alpinum		-2.3	-0.8	-1.3		-0.5	-2.5	0.0
Hedysarum boreale	-3.8	-18.6		-1.5	-3.3	-0.8	-1.0	-8.5
Lupinus arcticus	-0.8							
Mertensia paniculata	0.1		-6.0		-0.3	0.4	-0.1	
Pyrola Spp.		0.0					0.3	
Senecio spp.		-0.1					-3.4	
Solidago spp.	-1.8	-0.2	0.1	0.3	-1.1	-1.1	0.7	-0.4
Zygadenus elegans	-0.1	-0.3		-1.3	1.3	-0.3	-4.0	
Graminoids								
Bromus pumpellianus	0.3		0.5			1.5	-1.8	2.3
Calamagrostis	-0.9	-14.8	-5.3	-23.4	-4.3	-7.4	-4.8	-9.9
purpurascens	2.5	1.4	0.2	0.0	0.0	1.0	0.4	0.6
Carex spp.	-3.5	-1.4	-0.3	0.8	0.9	-1.0	-0.4	-0.6
Elymus trachycaulus				2.0	2 7		-0.3	5.0
Luzula arcticus	a a		5.0	-3.8	-2.5	1.0		-5.0
Poa glauca	-2.8		-5.0		-0.8	-1.3	0.2	-4.5
Seed Mix	0.3				1.9	1.7	0.3	

DAW								
Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Erect Shrubs (<1.5 m)								
Rhododendron	3.0	26	15	-8.0	6.0	-0.8	25	_1.8
groenlandicum	5.0	2.0	1.5	-8.0	0.0	-0.8	2.5	-1.0
Rosa acicularis	-1.1	-2.0	-3.3	-7.8	1.1	0.0	-0.6	-0.4
Rubus idaeus	-1.8	0.1	-0.6	-0.8	-1.9	0.0	-0.1	-0.1
Spirea beauverdiana	-5.0							
Vaccinium uliginosum	-6.0	-13.2	5.9	-16.4	17.4	0.8	1.3	-1.4
Prostrate Shrubs								
Arctostaphylos uva-ursi	0.1	0.7	3.6	0.1	1.1	-4.4	0.8	-0.3
Diphasiastrum complanatum					-1.8			4.3
Linnaea borealis	-20.3	0.9	2.5	-0.7	-0.6	-0.5	-1.0	0.1
Vaccinium vitis-idaea	-3.3	-1.6	-0.6	-2.2	-3.4	-2.6	-0.4	-1.4
Forbs								
Achillea millefolium	-0.8	-0.1	0.8	1.2	-0.1	1.9	-0.4	-0.1
Antennaria spp.	-1.0	-0.3	-3.8	-1.0	-0.3	-2.9	0.5	-0.5
Arnica angustifolia	1.5	0.5	0.8	0.1	0.2	0.3	0.6	0.1
Chamerion angustifolium	-2.9	-3.1	-0.5	-3.6	-2.3	-1.7	-3.2	-3.2
Cornus canadensis	-9.4	-8.1	-7.3	-17.4	3.6	0.8	-3.6	0.2
Equisetum arvense/pratense	-6.8	-1.3	-3.1	-1.3	-1.9	-1.4	-0.4	-5.9
Equisetum scirpoides	-1.1	0.3		0.3			0.1	-0.1
Geocaulon lividum	0.0	-1.3		-2.8	0.0			0.3
Lupinus arcticus	-2.4	-0.3	0.0	-0.2	-1.5	1.2	0.0	-12.3
Mertensia paniculata	-1.6	-0.6	-0.1	0.1	0.0	-0.8	-0.4	-1.6
Senecio spp.	1.8	0.4	0.4	-0.1	0.8	3.1	0.4	-0.3
Solidago spp.			0.1	3.1	0.1	0.5	0.3	
Taraxacum ceratophorum	-1.9	-0.9	-0.6	-0.8	0.8	1.0	-9.9	-3.0
Trifolium hybridum						-3.4		
Graminoids								
Calamagrostis canadensis	0.8	1.8	0.8	0.3	0.8	1.5	0.8	0.8
Carex spp.				-0.4		0.3	1.3	-0.8
Elymus trachycaulus								1.5
Festuca altaica	-0.9	2.0	5.8	-5.8	-0.6	-6.4	0.5	1.3
Festuca saximontana							1.3	
Seed Mix					1.9	1.8	1.6	

HJ1								
Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Erect Shrubs (<1.5 m)								
Rosa acicularis	-1.1	-4.4	-0.1	-5.5		0.1		-3.3
Shepherdia canadensis	0.5		-1.3			0.3		
Prostrate Shrubs								
Arctostaphylos uva-ursi	-41.5	-7.0	3.3	2.4	0.4	-1.3	-6.7	-13.3
Linnaea borealis			0.0					
Vaccinium vitis-idaea	-6.1	5.4			2.0	0.8	-2.5	2.8
El								
		0.1	20	1.0	0.1	1 1	0.1	0.1
Achillea millefolium	0.6	-0.1	-3.8	1.9	-0.1	-1.1	-0.1	0.1
Anemone spp.	-0.6	-3.3	0.3	0.3	0.0	1 1		-0.3
Astragalus spp.	-0.1	2.0	-0.8	-2.8	-0.3	-1.1		1 7
Chamerion angustifolium	-0.5	-3.8	-7.5	0.3	2.8	-1.4	0.0	-1.5
Equisetum scirpoides	0.0	0.9		0.3	0.4	0.3	-0.8	1.0
Eurybia siberica			-0.3			-1.8		3.1
Fragaria virginiana	0.1							
Geocaulon lividum	-2.3	-1.3		0.3		0.0	-2.5	
Hedysarum alpinum	-0.5	-0.8	-0.2		0.3			0.3
Pyrola Spp.				0.3				
Senecio spp.	0.5		3.6		0.8	1.6	0.3	0.5
Solidago spp.	-1.0	0.3	-2.2	-0.2	1.5	3.3	-0.2	-1.4
Zygadenus elegans		-0.6	-0.8	0.8		-0.4		
C 1								
Graminoids	0.4	7 7	1.0	0.2	0.5	1.0	2.4	4.0
Bromus pumpellianus	0.4	-/./	-1.0	0.3	0.5	-1.2	-2.4	-4.8
Calamagrostis	0.6	-8.2	4.1	0.7	-0.3	-2.3	-0.8	1.0
purpurascens	0.6	07	0.2	0.6	0.1	1.2		0.1
Carex spp.	0.6	-0.7	0.3	-0.6	0.1	-1.3		0.1
Elymus trachycaulus	0.1		23.8	0.1	0.5	3.8	0.0	
Festuca saximontana	0.1		2.1	0.1	0.5	0.0	-0.3	
Trisetum spicatum	0.1			0.5		~ -		
Seed Mix					0.8	0.5	0.1	

HJ2								
Treatment	BS-T	BS-I	CS-T	CS-I	PI-T	PI-I	SC	MC
Erect Shrubs (<1.5 m)								
Rosa acicularis	-1.0	-5.6	-5.1	-10.3	-1.0	-1.4	-1.1	-0.3
Shepherdia canadensis	-7.4	-10.6	-0.5	-11.4	-1.3	-9.6	5.0	-0.5
Prostrate Shrubs								
Arctostaphylos uva-ursi	5.0	-0.9	2.3	20.0	-21.9	-7.8	-1.5	-0.5
Juniperus horizontalis							1.3	-5.0
Linnaea borealis	-6.1	-0.1	0.0	-1.9	1.5	2.8	0.6	8.8
Vaccinium vitis-idaea	0.3		-1.3					
Forbs								
Achillea millefolium	-0.5	-1.5	-0.3	-2.2	0.2	-0.1	-0.6	-1.1
Anemone spp.	-1.0	-1.3	0.4	-1.6	-0.3	-1.5	-0.3	0.6
Antennaria spp.	-0.3	-5.5		-0.8		0.8	0.1	-0.3
Astragalus spp.	-0.3	-18.8		0.1	-0.3		-0.3	
Chamerion angustifolium	-3.5	-4.6	-1.9	-1.9	-0.9	-1.5	-2.5	-2.4
Equisetum			0.1		-0.3	0.3		-0.1
arvense/pratense					0.0	0.0		011
Equisetum scirpoides	-0.8	0.4	0.3	1.3	-0.5	0.1	-0.1	
Eurybia siberica	-0.3			-1.9	0.0		0.5	-0.3
Fragaria virginiana	-6.0	-3.8	0.3	-0.3	-4.9	-2.1	-0.1	-1.5
Hedysarum alpinum		-0.5			0.3	-4.5		
Lupinus arcticus	-0.9							
Mertensia paniculata	-0.1	-0.3	-0.1	-1.0	0.0	0.9	0.5	
Pyrola Spp.		-0.3	0.3					1.1
Senecio spp.		0.2	-0.4	0.6	0.1	0.0	-0.7	-0.2
Solidago spp.	-1.6	-0.3	0.2	-1.7	-0.3	2.9	-0.3	-0.9
Graminoids								
Bromus pumpellianus			-0.6	-3.0				0.0
Calamagrostis	4.2	-7.8	-2.3	-5.1	-1.9	-21.4	-1.3	-5.0
purpurascens	2.2	0.0	0.4	0.0	0.6	1 1	0.1	0.0
Carex spp.	-3.2	-0.9	-0.4	-0.8	-0.0	-1.1	0.1	-0.8
Elymus trachycaulus	0.7	-4.3	1.5	-1.2	0.1	0.6	0.8	0.8
Festuca altaica	0.2	0.1	-1.5	-0.3	1.5	-0.5	0.6	4.9
r estuca saximontana	-0.2	-2.1	0.1	-1.4	-0.3	-3.8	-2.0	-1.3
<i>i risetum spicatum</i>			0.5		0.3	25	1.0	
Seed Mix			0.1		5.5	2.5	1.2	

APPENDIX 3: R coding for statistical analysis of dose-response relationships

library(drc)

#Four parameter Weibull model1<-drm(Endpoint~ug.g,data=Data,subset=Species=="",fct=W2.4(),na.action=na.omit)

#Three parameter Weibull model1<-drm(Endpoint~ug.g,data=Data,subset=Species=="",fct=W2.3(),na.action=na.omit)

shapiro.test(resid(model1))
#p value should be >0.05

library(car)
leveneTest(resid(model1)~as.factor(ug.g),data=Data[Data\$Species=="",])
#p value should be >0.05

#if model residuals fail one or both of the tests, try boxcox transformation of both sides model1b<-boxcox(model1,method="anova")

shapiro.test(resid(model1b))
#p value should be >0.05
#if not, look at QQ to see distribution visually

qqnorm(resid(model1b)) qqline(resid(model1b)) #sometimes a few outliers cause failure of Shapiro-Wilk Test, but the majority of residuals follow a normal distribution and model assumptions are not violated

library(car)
leveneTest(resid(model1b)~as.factor(ug.g),data=Data[Data\$Species=="",])
#p value should be >0.05
#if this didn't improve after boxcox there may be issues with the model itself
#can check residuals visually for clues

plot(fitted(model1b),resid(model1b))

#Once satisfied with the model, run a lack of fit test modelFit(model) #p value should be >0.05 #not a strong test, if model fails, do a visual check to see if there is actually a lack of fit plot(model,type="all") #not convinced the modelFit test is appropriate after a boxcox transformation as it always seems to decrease

#Calculate ED values ED(model,c(10,25,50))

APPENDIX 4: Parameters and EC/IC estimates for seedling emergence and seedling growth test

Dose response curve model parameters and inhibition concentration estimates for the 28 day seedling emergence and seedling growth tests determining the toxicity of triclopyr in five northern soils to *A. millefolium* (ACHIMI), *C. canadensis* (CALACA) and *C. angustifolium* (CHAMAN). Soil (CAR, DAW, HJ1, HJ2, LS) characteristics and locations are described in Table 4.2. (Chapter 4). Inclusion of f parameter indicates Brain-Cousens hormesis model was used, otherwise a Weibull function was used. Missing *C. angustifolium* shoot/root endpoints are due to inadequate germination for modelling and biomass data were not modelled due to extreme variability across sites and doses. SE refers to the standard error of the mean.

Soil Species		End		Model Par	rameters			Inhibition Concentration Estimates			
5011	Species	Point	$b \pm SE$	d ±SE	e ±SE	f ±SE	n	IC10 ± SE	IC25 ±SE	IC50 ±SE	
CAR	ACHIMI	Shoot	-0.57 ±0.07	28.25 ± 2.58	2.82 ±1.00		40	0.67 ±0.34	1.61 ±0.65	5.32 ±1.65	
CAR	ACHIMI	Roots	-1.52 ±0.16	163.47 ± 15.73	3.19 ±0.63		40	1.85 ±0.45	2.58 ±0.55	4.07 ±0.73	
CAR	ACHIMI	Biomass	-0.68 ± 0.08	3.91 ±0.64	1.26 ±0.59		40	0.37 ±0.22	0.78 ±0.40	2.15 ±0.92	
CAR	CALACA	Shoot	-0.90 ±0.19	76.07 ±4.14	6.02 ±1.16		43	2.38 ±0.72	4.18 ±0.93	9.05 ±1.77	
CAR	CALACA	Roots	-1.17 ±0.13	127.86 ±21.05	2.30 ±0.70		43	1.12 ±0.41	1.74 ±0.57	3.15 ±0.88	
CAR	CALACA	Biomass	-0.88 ±0.13	3.22 ±0.53	2.88 ±1.13		43	1.12 ±0.55	1.99 ±0.86	4.36 ±1.56	
CAR	CHAMAN	Shoot	0.64 ±0.41	20.50 ±3.35	4.90 ±3.58		25	0.15 ±0.31	0.72 ±0.85	2.79 ±1.95	
DAW	ACHIMI	Shoot	-0.90 ±0.12	41.39 ±5.85	3.12 ±1.12		36	1.24 ±0.57	2.17 ±0.85	4.69 ±1.50	
DAW	ACHIMI	Roots	-2.61 ±0.38	164.43 ± 13.43	4.36 ±0.58		35	3.16 ±0.54	3.85 ±0.57	5.02 ±0.60	
DAW	ACHIMI	Biomass	-1.60 ±0.34	6.87 ±1.22	3.27 ±0.95		36	1.95 ±0.72	2.67 ±0.86	4.11 ±1.08	
DAW	CALACA	Shoot	-0.71 ±0.08	122.78 ± 17.98	2.24 ±0.95		35	0.69 ±0.37	1.41 ±0.66	3.76 ±1.45	
DAW	CALACA	Roots	1.75 ±0.26	120.32 ±24.79	2.70 ±0.71	5.37 ±6.04	36	0.94 ±0.47	1.68 ±0.62	3.11 ±0.90	
DAW	CALACA	Biomass	-1.12 ±0.16	6.42 ±1.90	2.04 ±1.00		36	0.97 ±0.55	1.52 ±0.79	2.83 ±1.30	
HJ1	ACHIMI	Shoot	1.42 ±0.07	19.83 ±3.37	0.76 ±1.20	34.38 ±79.42	39	1.99 ±1.16	3.17 ±1.60	8.02 ±3.61	
HJ1	ACHIMI	Roots	-1.98 ±0.36	184.64 ±21.52	$4.26 \hspace{0.2cm} \pm \hspace{-0.2cm} 0.88$		39	2.80 ±0.75	3.61 ±0.83	5.13 ±0.94	
HJ1	ACHIMI	Biomass	-0.92 ±0.23	4.32 ±0.82	$2.29 \hspace{0.2cm} \pm 1.19$		39	0.92 ±0.65	1.60 ±0.94	3.41 ±1.55	
HJ1	CALACA	Shoot	-0.73 ±0.13	73.16 ±9.59	7.54 ±2.93		40	2.39 ±1.22	4.81 ±2.06	12.47 ±4.50	
HJ1	CALACA	Roots	-1.09 ±0.13	149.32 ±38.91	2.20 ±0.99		41	1.02 ±0.53	1.63 ±0.78	3.08 ±1.30	
HJ1	CALACA	Biomass	-0.77 ±0.13	3.62 ±1.03	3.62 ±2.34		40	1.23 ±0.95	2.38 ±1.64	5.82 ±3.48	

Model Parameters	s and IC	Estimates	Continued
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species			Model Parameters				Inhibition Concentration Estimates			
species	Point	$b \pm SE$	d ±SE	e ±SE	f ±SE	п	IC10 ± SE	IC25	±SE IC5	±SE
ACHIMI	Shoot	-1.31 ±0.59	38.41 ±5.45	5.13 ±2.21		31	2.71 ±1.89	4.00	±2.13 6.7	±2.23
ACHIMI	Roots	-3.55 ±1.21	141.37 ± 13.02	7.28 ± 0.99		31	5.75 ±1.09	6.64	±1.02 8.0	±0.97
ACHIMI	Biomass	-2.06 ±0.39	8.71 ±1.35	3.67 ± 0.89		32	2.45 ±0.76	3.14	±0.84 4.39	±0.94
CALACA	Shoot	-0.70 ±0.13	76.24 ± 9.00	8.21 ±4.86		44	1.93 ±1.56	4.66	±3.10 15.5	±8.34
CALACA	Roots	2.27 ±0.14	77.43 ± 18.49	2.04 ± 1.06	86.71 ±93.02	45	4.29 ±1.23	5.03	±1.36 7.0	±1.73
CALACA	Biomass	-0.91 ±0.19	5.09 ± 1.15	2.10 ± 1.04		45	0.85 ±0.53	1.47	±0.80 3.12	±1.40
CHAMAN	Shoot	-2.18 ±0.98	66.84 ± 3.49	2.34 ± 0.30		24	1.60 ±0.38	2.02	±0.32 2.7	±0.35
CHAMAN	Roots	-2.65 ±2.31	210.01 ± 15.00	2.18 ±0.42		24	1.59 ±0.69	1.93	±0.54 2.50	±0.35
ACHIMI	Shoot	-0.56 ±0.20	11.44 ±0.70	19.88 ±9.07		37	4.55 ±2.83	11.16	±5.18 38.0	±20.66
ACHIMI	Roots	-0.94 ±0.35	102.39 ±8.27	6.54 ±2.38		37	2.69 ±1.54	4.62	±1.97 9.68	±3.27
ACHIMI	Biomass	-0.67 ±0.27	1.31 ± 0.09	13.97 ±5.95		37	4.06 ±2.68	8.61	±4.05 24.04	±11.48
CALACA	Shoot	-1.11 ±0.16	$6.42 \hspace{0.2cm} \pm 1.90$	2.04 ± 1.00		41	5.48 ±2.95	11.24	±5.12 29.9	±10.54
CALACA	Roots	-1.30 ±0.17	108.08 ± 15.45	4.52 ±1.39		41	2.39 ±0.90	3.52	±1.17 5.98	±1.67
CALACA	Biomass	-0.48 ±0.15	$0.97 \hspace{0.2cm} \pm 0.15$	10.54 ±8.12		41	1.92 ±2.23	5.41	±4.91 22.23	±14.75
CHAMAN	Shoot	-0.51 ±0.19	$7.30 \hspace{0.1in} \pm 0.87$	7.97 ±4.43		30	1.58 ±1.43	4.23	±2.75 16.2	±9.05
CHAMAN	Roots	-1.08 ±0.43	20.30 ±2.73	3.86 ±1.63		30	1.78 ± 1.00	2.85	±1.30 5.42	±2.29
	ACHIMI ACHIMI ACHIMI CALACA CALACA CALACA CHAMAN ACHIMI ACHIMI CALACA CALACA CALACA CALACA	ACHIMIShootACHIMIRootsACHIMIBiomassCALACAShootCALACARootsCALACABiomassCHAMANShootCHAMANShootACHIMIShootACHIMIBiomassACHIMIBiomassCALACABiomassCALACABiomassCALACABiomassCALACABiomassCALACAShootCALACABiomassCALACABiomassCHAMANShoot	ACHIMI Shoot -1.31 ±0.59 ACHIMI Roots -3.55 ±1.21 ACHIMI Biomass -2.06 ±0.39 CALACA Shoot -0.70 ±0.13 CALACA Roots 2.27 ±0.14 CALACA Biomass -0.91 ±0.19 CHAMAN Shoot -2.65 ±2.31 CHAMAN Roots -2.65 ±2.31 ACHIMI Biomass -0.94 ±0.35 ACHIMI Roots -0.67 ±0.27 CALACA Shoot -0.56 ±0.20 ACHIMI Roots -0.94 ±0.35 ACHIMI Biomass -0.67 ±0.27 CALACA Shoot -1.11 ±0.16 CALACA Biomass -0.648 ±0.15 CALACA Biomass -0.48 ±0.15 CALACA Biomass -0.51 ±0.19 CALACA Shoot -1.130 ±0.17 CALACA Biomass -0.51 ±0.19 CHAMAN Shoot -0.51 ±0.19	ACHIMI Shoot -1.31 ±0.59 38.41 ±5.45 ACHIMI Roots -3.55 ±1.21 141.37 ±13.02 ACHIMI Biomass -2.06 ±0.39 8.71 ±1.35 CALACA Shoot -0.70 ±0.13 76.24 ±9.00 CALACA Roots 2.27 ±0.14 77.43 ±18.49 CALACA Biomass -0.91 ±0.19 5.09 ±1.15 CHAMAN Shoot -2.65 ±2.31 210.01 ±15.00 ACHIMI Biomass -0.56 ±0.20 11.44 ±0.70 ACHIMI Roots -0.67 ±0.27 1.31 ±0.09 CALACA Shoot -0.56 ±0.20 11.44 ±0.70 ACHIMI Roots -0.94 ±0.35 102.39 ±8.27 ACHIMI Biomass -0.67 ±0.27 1.31 ±0.09 CALACA Shoot -1.11 ±0.16 6.42 ±1.90 CALACA Shoot -1.30 ±0.17 108.08 ±15.45 CALACA Biomass -0.48 ±0.15 0.97 ±0.15 CALACA Biomass -0.48 ±0.15 0.97 ±0.15 CALACA Biomass -0.51 ±0.19 7.30 ±0.87 CHAMAN Shoot <td>ACHIMIShoot$-1.31 \pm 0.59$$38.41 \pm 5.45$$5.13 \pm 2.21$ACHIMIRoots$-3.55 \pm 1.21$$141.37 \pm 13.02$$7.28 \pm 0.99$ACHIMIBiomass$-2.06 \pm 0.39$$8.71 \pm 1.35$$3.67 \pm 0.89$CALACAShoot$-0.70 \pm 0.13$$76.24 \pm 9.00$$8.21 \pm 4.86$CALACARoots$2.27 \pm 0.14$$77.43 \pm 18.49$$2.04 \pm 1.06$CALACABiomass$-0.91 \pm 0.19$$5.09 \pm 1.15$$2.10 \pm 1.04$CHAMANShoot$-2.18 \pm 0.98$$66.84 \pm 3.49$$2.34 \pm 0.30$CHAMANRoots$-2.65 \pm 2.31$$210.01 \pm 15.00$$2.18 \pm 0.42$ACHIMIShoot$-0.56 \pm 0.20$$11.44 \pm 0.70$$19.88 \pm 9.07$ACHIMIRoots$-0.94 \pm 0.35$$102.39 \pm 8.27$$6.54 \pm 2.38$ACHIMIBiomass$-0.67 \pm 0.27$$1.31 \pm 0.09$$13.97 \pm 5.95$CALACAShoot$-1.11 \pm 0.16$$6.42 \pm 1.90$$2.04 \pm 1.00$CALACARoots$-1.30 \pm 0.17$$108.08 \pm 15.45$$4.52 \pm 1.39$CALACABiomass$-0.67 \pm 0.27$$1.31 \pm 0.09$$13.97 \pm 5.95$CALACARoots$-1.30 \pm 0.17$$108.08 \pm 15.45$$4.52 \pm 1.39$CALACABiomass$-0.48 \pm 0.15$$0.97 \pm 0.15$$10.54 \pm 8.12$CHAMANShoot$-0.51 \pm 0.19$$7.30 \pm 0.87$$7.97 \pm 4.43$CHAMANRoots$-1.08 \pm 0.43$$20.30 \pm 2.73$$3.86 \pm 1.63$</td> <td>ACHIMIShoot-1.31 ±0.5938.41 ±5.455.13 ±2.21ACHIMIRoots-3.55 ±1.21141.37 ±13.027.28 ±0.99ACHIMIBiomass-2.06 ±0.398.71 ±1.353.67 ±0.89CALACAShoot-0.70 ±0.1376.24 ±9.008.21 ±4.86CALACABiomass-0.91 ±0.195.09 ±1.152.10 ±1.0486.71 ±93.02CALACABiomass-0.91 ±0.195.09 ±1.152.10 ±1.04CHAMANShoot-2.18 ±0.9866.84 ±3.492.34 ±0.30CHAMANRoots-2.65 ±2.31210.01 ±15.002.18 ±0.42ACHIMIShoot-0.56 ±0.2011.44 ±0.7019.88 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1.89 4.00 ± 2.13 6.79 ACHIMIRoots -3.55 ± 1.21 141.37 ± 13.02 7.28 ± 0.99 $ 31$ 5.75 ± 1.09 6.64 ± 1.02 8.07 ACHIMIBiomass -2.06 ± 0.39 8.71 ± 1.35 3.67 ± 0.89 $ 32$ 2.45 ± 0.76 3.14 ± 0.84 4.39 CALACAShoot -0.70 ± 0.13 76.24 ± 9.00 8.21 ± 4.86 $ 44$ 1.93 ± 1.56 4.66 ± 3.10 15.51 CALACARoots 2.27 ± 0.14 77.43 ± 18.49 2.04 ± 1.06 86.71 ± 93.02 45 4.29 ± 1.23 5.03 ± 1.36 7.01 CALACABiomass -0.91 ± 0.19 5.09 ± 1.15 2.10 ± 1.04 $ 45$ 0.85 ± 0.53 1.47 ± 0.80 3.13 CHAMANShoot -2.18 ± 0.98 66.84 ± 3.49 2.34 ± 0.30 $ 24$ 1.60 ± 0.38 2.02 ± 0.32 2.77 CHAMANShoot -2.65 ± 2.31 21.001 ± 15.00 2.18 ± 0.42 $ 24$ 1.60 ± 0.38 2.02 ± 0.32 2.77 CHAMANRoots -2.65 ± 2.31 21.001 ± 15.00 2.18 ± 0.42 $ 24$ 1.60 ± 0.38 2.02 ± 0.32 2.77 CHAMANRoots -0.66 ± 0.20 11.44 ± 0.70 19.88 ± 9.07 $ 37$ 4.55 ± 2.83 11.16 ± 5.18 38.01 ACHIMI

APPENDIX 5: Triclopyr dose-response curves for seedling emergence and seedling growth test

Dose response curves for 28 day Seedling Emergence and Seedling Growth Test assessing the inhibitory effects of triclopyr concentrations in soil on the early growth of *A. millefolium, C. canadensis*, and *C. angustifolium*.

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Figure A5.3. Response of *C. angustifolium* shoot length to triclopyr concentrations in CAR soil in 28 day seedling emergence and seedling growth test.



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Figure A5.18. Response of *A. millefolium* mean plant dry biomass to triclopyr concentrations in HJ1 soil in 28 day seedling emergence and seedling growth test.



Figure A5.19. Response of *C. canadensis* mean plant dry biomass to triclopyr concentrations in HJ1 soil in 28 day seedling emergence and seedling growth test.



Concentration of Theopyr In Con (ug/g)

Figure A5.20. Response of *A. millefolium* shoot length to triclopyr concentrations in HJ2 soil in 28 day seedling emergence and seedling growth test.



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Figure A5.35. Response of *C. canadensis* mean plant dry biomass to triclopyr concentrations in LS soil in 28 day seedling emergence and seedling growth test.



Concentration of Triclopyr in Soil (ug/g)

APPENDIX 6: IC estimates for toxicity tests following Environment Canada's statistical analysis methods

In addition to the analyses presented in Chapter 4, vegetative vigour and seedling emergence and seedling growth test data were also analyzed following the protocol "Point estimate for quantitative sublethal tests" in:

Environment Canada. 2005. Guidance document: Statistical methods for environmental toxicity tests. Environmental Protection Series, EPS1/RM/46. Ottawa, ON.

A Weibull function was used for non-linear regression modelling, as recommended by a statistician (E. Lamb, pers. comm.). Point estimates are summarized in below.

Species	Herbicide	Endpoint	Analysis Method	IC10 (%)	IC25 (%)	IC50 (%)
ACHI	Imazapyr	Aboveground Biomass	Linear Interpolation	1.14	1.39	1.77
ACHI	Triclopyr	Aboveground Biomass	Non-Linear Regression	0.63	3.30	31.87
CHAM	Imazapyr	Aboveground Biomass	Non-Linear Regression	0.02	0.12	1.15

Seedling Emergence and Seedling Growth Test (triclopyr only)

Species	Soil	Endpoint	Analysis Method	IC10 (ug/g)	IC25 (ug/g)	IC50 (ug/g)
ACHI	CAR	Root Length	Linear Interpolation	2.22	3.34	5.66
ACHI	CAR	Shoot Length	Non-Linear Regression	0.67	1.61	5.32
ACHI	CAR	Total Biomass	Linear Interpolation	1.88	3.23	4.52
ACHI	DAW	Root Length	Linear Interpolation	3.04	3.82	5.52
ACHI	DAW	Shoot Length	Linear Interpolation	1.24	1.71	7.44
ACHI	DAW	Total Biomass	Linear Interpolation	1.12	1.33	1.77
ACHI	HJ1	Root Length	Linear Interpolation	2.39	3.73	6.73
ACHI	HJ1	Shoot Length	Linear Interpolation	2.70	4.40	9.81
ACHI	HJ1	Total Biomass	Linear Interpolation	1.54	2.34	5.90
ACHI	HJ2	Root Length	Linear Interpolation	5.30	6.40	8.78
ACHI	HJ2	Shoot Length	Linear Interpolation	1.70	3.54	11.12
ACHI	HJ2	Total Biomass	Linear Interpolation	3.72	4.15	5.53
ACHI	LS	Root Length	Linear Interpolation	3.32	3.98	12.60
ACHI	LS	Shoot Length	Linear Interpolation	3.81	4.60	18.61
ACHI	LS	Total Biomass	Non-Linear Regression	4.06	8.61	24.04
CALA	CAR	Root Length	Linear Interpolation	1.90	5.92	7.68
CALA	CAR	Shoot Length	Linear Interpolation	4.16	5.64	8.68
CALA	CAR	Total Biomass	Linear Interpolation	4.29	5.31	7.33
CALA	DAW	Root Length	Linear Interpolation	4.96	5.64	7.43
CALA	DAW	Shoot Length	Linear Interpolation	3.88	4.35	5.27

Species	Soil	Endpoint	Analysis Method	IC10 (ug/g)	IC25 (ug/g)	IC50 (ug/g)
CALA	DAW	Total Biomass	Linear Interpolation	4.44	5.11	6.82
CALA	HJ1	Root Length	Linear Interpolation	1.40	2.30	9.18
CALA	HJ1	Shoot Length	Linear Interpolation	7.23	8.04	9.80
CALA	HJ1	Total Biomass	Linear Interpolation	6.85	7.50	8.73
CALA	HJ2	Root Length	Linear Interpolation	6.76	7.79	10.51
CALA	HJ2	Shoot Length	Linear Interpolation	8.50	9.84	12.57
CALA	HJ2	Total Biomass	Linear Interpolation	3.81	4.22	5.98
CALA	LS	Root Length	Linear Interpolation	2.84	3.74	6.96
CALA	LS	Shoot Length	Linear Interpolation	3.16	3.70	4.82
CALA	LS	Total Biomass	Linear Interpolation	4.29	7.48	13.42
CHAM	CAR	Shoot Length	Linear Interpolation	1.32	1.90	2.70
CHAM	HJ2	Root Length	Non-Linear Regression	1.59	1.93	2.50
CHAM	HJ2	Shoot Length	Non-Linear Regression	1.60	2.02	2.77
CHAM	LS	Root Length	Non-Linear Regression	1.78	2.85	5.43
CHAM	LS	Shoot Length	Linear Interpolation	2.82	3.06	3.51

Seedling Emergence and Seedling Growth Test (triclopyr only) Continued

APPENDIX 7: Methods for determination of soil characteristics

Determination of Bulk Density

- 1. Remove litter from soil surface with a rake
- 2. Drive small metal cylindrical ring (inner diameter of 4.0 cm) into soil with a mallet and wooden block until resistance changes (transition between organic and first mineral layer)
- 3. Record depth of ring from outer edge to soil surface
- 4. Remove ring by slicing soil with a steak knife around the edges, removing soil on one side of the ring and slicing horizontally underneath to separate bottom of ring from soil
- 5. Slide knife carefully under ring and tilt ring horizontally in smooth motion to ensure soil does not fall out of ring
- 6. Remove excess soil with knife
- 7. Place soil in plastic Ziploc bag and seal for transport back to lab
- 8. Collect two more samples in same manner for a total of three replicates
- 9. Transfer soil from plastic bag to tin pie plate and weigh to nearest mg; recorded fresh weight
- 10. Dry soil in oven at 105°C for 24 hours
- 11. Weigh dried soil to nearest mg
- 12. Calculate bulk density (g/cm^3) with the equation:

 $\frac{\text{dry weight of sample}}{\pi(\text{ring diameter/2})^2 \text{ x (total length of ring - depth of ring from outer edge to soil surface)}}$

13. Average bulk density per sample to calculate bulk density for soil type

Determination of pH

- 1. Sieve each soil sample to 2 mm
- 2. Weigh out five replicates of 4 g ± 0.05 g sub-samples into glass test tubes
- 3. Add 20 mL of 0.1% CaCl₂ solution to each sample and apply lids to test tubes
- 4. Shake for 30 min
- 5. Let stand for 60 minutes
- 6. Calibrate pH meter with pH 4, 7 and 10 calibration solutions
- 7. Place pH probe in test tube until pH meter indicates steady reading
- 8. Record pH

Determination of Total Nitrogen

Total nitrogen was determined by combustion analysis with a LECO-CNS 2000 (LECO Corp.,

St. Joseph, MI).

Sample Prep:

- 1. Air dry soil samples for 48 hours
- Grind each soil sample to very find powder with Reutsch ZM200 plant grinder at 14,000 RPM
- 3. Use a 3 g subsample to determine percent moisture in Mettler Toledo MJ33 Moisture Analyzer (Mettler Toledo Canada, Mississsauga, ON) for each soil sample
- 4. Weigh 200 mg ± 10 of each subsample (5 replicates per soil) into ceramic crucible and record weight to 0.1 mg

Analysis:

- 1. Set LECO-CNS 2000 for plant tissue analysis as samples contained high amounts of organic material
- 2. Run 3 blank samples
- 3. Run 3 samples with standard 502-274 wheat flour for calibration
- 4. Run a QC sample
- 5. Run 20 samples
- 6. Repeat steps 4 and 5 until completion

Calculations:

- 1. Percent Total Nitrogen per sample = Percent Total Nitrogen from Analysis/(100-Percent Moisture)
- 2. Calculate the mean and standard error of five replicates for percent total nitrogen of soil

Determination of Total Organic Carbon

Total organic carbon was determined by combustion analysis with a LECO-C632 (LECO Corp.,

St. Joseph, MI).

Sample Prep:

- 1. Air dry soil samples for 48 hours
- Grind each soil sample to very find powder with Reutsch ZM200 plant grinder at 14,000 RPM
- 3. Use a 3 g subsample to determine percent moisture in Mettler Toledo MJ33 Moisture Analyzer (Mettler Toledo Canada, Mississsauga, ON) for each soil sample
- 4. Weigh 200 mg ± 10 of each subsample (5 replicates per soil) into ceramic crucible and record weight to 0.1 mg

Carbonate Removal

1. Wet each samples with approximately 1 mL of deionized water

- 2. Place samples in a dessicator with three 150 mL open containers each containing 50 mL of 12M HCl
- 3. Expose samples to fumes for 48 hours
- 4. Place samples in drying oven at 105°C overnight to remove residual moisture and HCl

Analysis (LECO-C632):

- 1. Run two blank samples prior to analysis
- 2. Run three replicates of LECO Standard #502-309 to calibrate
- 3. Run a QC sample
- 4. Run 20 samples
- 5. Repeat steps 4 and 5 until completion

Calculations:

- 1. Percent Total Organic Carbon per sample = Percent Carbon from Analysis/(100-Percent Moisture)
- 2. Calculate the mean and standard error of five replicates for percent total organic carbon of soil

APPENDIX 8: Dissipation of triclopyr from *Salix glauca* foliage over 30 days Introduction

The direct effects of imazapyr and triclopyr applications on Yukon right-of-way (ROW) plants and plant communities were the focus of this thesis, however persistence of these chemicals in plant tissue is also of concern. Plant tissues provide pathways for herbicide residue entry into the wider environment including transfer to wildlife (Tatum 2004). Foliage can also act as a source for soil contamination when fallen leaves decompose on the forest floor (Thompson et al. 1994). Imazapyr and triclopyr typically rapidly dissipate within 30 days of application, but some residues may persist for longer periods (Newton et al. 1990; Thompson et al. 1994; Newton et al. 2008).

It is widely accepted that herbicide dissipation rates from vegetation are significantly dependent on environmental conditions. On the leaf surface, the active ingredient can dissipate through volatilization, photolysis or microbial breakdown (Bentson and Norris 1991; Newton et al. 2008). The net effect of northern environmental conditions on these processes is unknown. Long summer photoperiods at higher latitudes may increase the photolysis rate on the leaf prior to herbicide absorption, however, microbial breakdown may be slowed by cooler temperatures. Once absorbed, plants can metabolize the active ingredient into less toxic metabolize or deposit contaminated leaves as litter (Newton et al. 1990). The ability to metabolize herbicides is typically species-specific (Sidhu and Feng 1993). Plant metabolic activity is slower in the North partially due to cool soil temperatures (Bonan and Shugart 1989) and may increase herbicide persistence in plant tissue. Ambient temperature also influences the dissipation rate from plant tissue. For example, rapid dissipation of triclopyr from foliage occurred for the first 80 days after application, but concentrations changed very little over the winter (Newton et al. 1990). In Alaska, however, Newton et al. (2008) found dissipation rates of triclopyr and imazapyr from vegetation similar to rates at lower latitudes.

To further investigate the dissipation kinetics of triclopyr in vegetation, a field experiment was conducted in southwestern Yukon during the months of June and July, 2015. The study focused on a single willow species, *Salix glauca*, with the objective of determining the dissipation time of triclopyr in *S. glauca* leaves over 30 days.

Material and Methods

Thirty *S. glauca* individuals were randomly selected in a 500 m section of ROW 80 km west of Whitehorse, YT on the Alaska Highway, $(60.778^{\circ}N, -136.071^{\circ}W)$. Individual *S. glauca* were a minimum of 2 m apart and at least 5 m from the edge of the ROW. Vascular plant species within a 1.5 m radius of the plant were removed by hand to reduce effects of competition between June 1st and the end of the experiment. Identification of *S. glauca* was conducted in early May to monitor catkin emergence and confirmed June 1st.

Herbicide treatments were applied with a backpack sprayer to a 1.5 x 1.5 m area around each individual at 4.530 kg a.i. ha⁻¹ triclopyr. Sampling was done within one hour of the spray drying and at 1, 3, 7, 14, and 30 days after spraying. Five *S. glauca* samples were harvested at each interval; each individual shrub was only sampled once. Each sample consisted of approximately 30 g of foliage collected in a Ziploc bag and frozen within 6 hours. Samples were analyzed by the University of Guelph Laboratory Services following certified method 069 Phenoxy Acid – Soil/Veg. Limits of detection ranged from 0.001 ppm to 0.005 ppm depending on amount of sample collected and recoveries ranged from 89.46% to 96.00%. A subsample was also used to determine percent moisture of each sample.

Prior to analysis, concentrations of triclopyr in fresh samples (ppm) were converted to μg g⁻¹ dry weight based on percent moisture.

A visual assessment of the triclopyr degradation relationship revealed an exponential decay pattern for the first seven days with a secondary peak at 14 DAT. An outlier at 7 DAT detected by Grubb's test in R package "outliers" (Lukasz 2011) was removed from the dataset. The value was >100 μ g g⁻¹ higher than the highest 0 DAT concentration and was likely caused by application error. A first order dissipation model was used to characterize the first seven days of dissipation (Equation A8.1). Triclopyr concentration data were log transformed to linearize the relationship prior to modelling. The secondary input of triclopyr between seven and 14 days after treatment prevented modelling of the entire period as the assumption of a stable initial concentration was violated.

Equation A8.1.

 $C_t = C_0 e^{(-kt)}$, where C_t is the concentration of triclopyr in foliage, C_0 is the initial concentration, *k* is the degradation rate constant for C_0 , and t is time after application.

The assumptions of normality and homogeneity of variance were assessed in QQ plots and fitted vs. residuals plots. To further interpret the secondary peak, concentrations of triclopyr were visually compared against precipitation and temperature data.

Linear regression analysis was completed in R version 3.1.2 using R package "stats" (R Core Team 2015).

Results and Discussion

The dissipation of triclopyr in *S. glauca* foliage over seven days was similar to patterns and rates reported elsewhere. The average initial triclopyr concentration in plant foliage was 136.58 μ g g⁻¹ and dissipated to a mean of 76.57 μ g g⁻¹ one week after treatment. (Figure A8.1.). A rapid decline in triclopyr residues with a DT₅₀ estimate between 1.5 and 2 days was expected (Whisenant and McArthur 1989; Thompson et al. 1994), however, our DT₅₀ estimate was 6.28 days. Average daily temperatures between 0-7 DAT ranged from 8-14°C and though daylight was >18 hrs (NRC n.d.), the cooler temperatures may have slowed triclopyr dissipation (Bentson and Norris 1991).



Figure A8.1. Dissipation of triclopyr residues in *Salix glauca* foliage on a Yukon power line right-of-way over a 30 day period between June 2^{nd} and July 2^{nd} , 2015. Dissipation during the first seven days followed a first order decay pattern (trendline) however a secondary input of triclopyr between seven and 14 days after treatment prevented modelling of the entire period as the assumption of a stable initial concentration was violated. Error bars indicate 95% confidence intervals around the mean.

The peak in residues at 14 DAT occurred shortly after the first substantial precipitation event during the study (2.6 mL at 9 DAT) and a dramatic increase in daily temperatures (Figure A8.2.). The combined effects temperature and precipitation are likely responsible for the secondary peak of triclopyr residue. Triclopyr residues in soil can increase after precipitation as unabsorbed active ingredient is washed off the vegetation (Thompson et al. 2000; Ranft et al. 2010). Sufficient soil moisture is required for plants to uptake herbicide in soil (Renner et al. 1988) and the warmer temperatures likely increased *S. glauca* metabolic activity resulting in soil uptake and rapid translocation of triclopyr into the foliage (Radosevich and Bayer 1979; Seiler et al. 1993).



Figure A8.2. Average daily temperature (°C) and total daily precipitation (mL) at a ROW research site, 80 km west of Whitehorse, YT, over a 30 day period between June 2nd and July 2nd, 2015.

Triclopyr in *S. glauca* foliage did not dissipate to low or undetectable levels by the end of our 30 day experiment. Research in Alaska demonstrated near complete dissipation of triclopyr from vegetation within 30-45 days (Newton et al. 2008) suggesting similar dissipation rates to southern studies (Whisenant and McArthur 1989, Thompson et al. 1994). Our results do not support that northern herbicide dissipation rates are similar to rates in warmer climates. Our triclopyr residues DT_{50} estimate was 6.28 days and concentrations at 7 DAT (mean=76.57 µg g⁻¹ ±2.49SE) were similar to concentrations at 30 DAT (mean=67.58 µg g⁻¹ ±9.23SE), likely due to secondary uptake of triclopyr from soil. Whether this phenomenon is linked to northern environmental conditions is unknown. Newton et al. (1990) also reported relatively high levels of triclopyr persisting in vegetation, however, their study included conifers with substantially different leaf morphology making comparisons difficult. *S. glauca* leaves were dried and shriveled at 30 DAT suggesting metabolic degradation of triclopyr residues had ceased. The deposition of leaves as litter will probably provide a secondary input of triclopyr residues into the soil (Tatum 2004; Thompson et al. 1994).

The persistent concentrations of triclopyr in S. glauca foliage are probably not high enough to cause acute toxicological responses in wildlife (Tatum 2004), however, the effects of chronic exposure are less certain. Acute toxicity testing with rats identified triclopyr as "practically nontoxic" (LD₅₀=630-729 mg/kg) (USEPA 1998). As a northern case study, S. glauca is a major component of moose (Alces alces) forage in the boreal forest during the spring and summer (McArt et al. 2009). Moose can ingest up to 38.4 g dry matter per kg body weight during peak mid-summer feeding (Renecker and Hudson 1985). In the worst case scenario of an individual foraging exclusively from contaminated S. glauca foliage and triclopyr concentrations remained stable at the initial concentrations (mean=136.58 μ g g⁻¹), a moose would consume 5.2 mg of triclopyr per kg body weight per day. If foliage contained triclopyr residues at 7 DAT concentrations (mean=76.57 μ g g⁻¹), moose daily intake of triclopyr would be 2.94 mg kg⁻¹ day⁻¹. Acute poisoning is therefore highly unlikely as maximum moose intake is more than 100 times lower than the rat LD₅₀ of 630 mg kg⁻¹, however, dietary and systemic tests may better represent toxicity of consumption over time. Dietary exposure tests identified no observed effect levels (NOELs) with rats (reproductive endpoints) at 5 mg kg⁻¹ day⁻¹, dogs (physiological endpoints) at 10 mg kg⁻¹ day⁻¹ and rabbits (reproductive endpoints) at 30 mg kg⁻¹ day⁻¹ (USEPA 1998). The Reference Dose (RfD) (formerly called Acceptable Dietary Intake) for triclopyr is 0.05 mg kg⁻¹ day⁻¹, 100 fold less than the lowest mammal NOEL to encompass inter- and intraspecies sensitivities (USEPA 1998). Moose consumption of sub-acutely toxic triclopyr residues in S. glauca foliage is therefore possible, but unlikely.

Between 7-30 DAT, reduction in forage quality could discourage moose from browsing as moose select for high quality forage during the summer (Van Beest et al. 2010). *S. glauca* foliage became limp after seven days with early signs of necrosis. Leaves were yellowing and drying out by 14 DAT and complete leaf death was recorded at 30 DAT. Triclopyr does not readily bioaccumulate (Carmichael et al. 1989), suggesting exposure would need to remain consistent to elicit toxic effects. Persistence of triclopyr in *S. glauca* woody tissue was beyond the scope of this study, but winter consumption of *S. glauca* shoots could also extend the exposure time of moose to residues. The length of exposure is therefore uncertain.

Conclusion

Dissipation of triclopyr residues was not a continuous or complete process within 30 days after application. Precipitation followed by warm daily temperatures likely facilitated secondary

uptake of triclopyr from the soil and increased triclopyr concentrations in foliage mid-way through the study period. *S. glauca* leaves deteriorated between 7-30 days after treatment suggesting long term browsing by ungulates such as moose is unlikely and initial triclopyr concentrations in foliage were well below acutely toxic levels. If continuous consumption of triclopyr contaminated foliage did occur, however, moose intake of triclopyr could exceed levels considered safe for daily ingestion.

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APPENDIX 9: Herbicide Damage Rating Scale – Yarrow (*Achillea millfolium*)

Damage Rating: 0



Damage Rating: 10



Damage Rating: 20



Damage Rating: 30



Damage Rating: 40



Damage Rating: 50


Damage Rating: 60



Damage Rating: 70



Damage Rating: 80



Damage Rating: 90



Damage Rating: 98



Damage Rating: 100

