LINKING HERBICIDE DISSIPATION TO SOIL ECOLOGICAL RISK ALONG RIGHT-OF-WAYS IN THE YUKON TERRITORY, CANADA

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ABSTRACT

In the Yukon Territory, vegetation management along transmission right-of-ways (ROWs) is conducted using brushing and mowing techniques alone. When cut, target species, such as *Populus spp*. and *Salix spp*., grow rapidly, shortening maintenance cycles. Long-term vegetation management may be improved by integrating herbicide application. However, prior to implementation, the dissipation and toxicity of herbicides in northern latitudes needed to be assessed. The dissipation of Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr) in soils was assessed at five ROW locations representative of the main ecoregion types where ROWs occur within the Yukon Territory.

Soils from four sites were collected to a depth of three centimetres at 1, 30 and 365 days after treatment (DAT) to determine dissipation of herbicides for each of three application methods (cut stump, point injection and backpack spraying). Soils from a fifth site were collected more frequently on days 0, 1, 3, 7, 14, 21, 30 and 60 to better determine the dissipation time of each herbicide in Yukon Territory soils. Mean triclopyr concentrations at 365 DAT were 0.01 ± 0.01 mg ai kg^{-1} and 0.24 ± 0.20 mg ai kg^{-1} for cut stump and point injection, respectively. Whereas, the mean concentrations for imazapyr cut stump and point injection treatments at 365 DAT were 0.01 ± 0.002 mg ai kg^{-1} and 0.03 ± 0.02 mg ai kg^{-1}, respectively. Dissipation rates for the backpack spray treatment indicated that triclopyr (time to 50% of the initial concentration [DT_{50}] of 1 DAT) dissipated faster than imazapyr (DT_{50} of 16 DAT). Residues from the cut stump and point injection treatments dissipated considerably between 30 and 365 DAT for both herbicides.

Soil dissipation data was linked to a series of standardized soil toxicity tests, including three soil invertebrate tests (*Enchytreaus crypticus*, *Folsomia candida,* and *Oppia nitens*) and three soil enzyme tests (arylsulfatase, B-glucosidase and phosphatase). Expected maximum application concentrations (75.5 mg triclopyr kg d.w.$^{-1}$ and 12 mg imazapyr kg d.w.$^{-1}$) were below the 28-day (28-d) EC_{25} for all species tested. Even sensitive endpoints such as 28-d LC_{10} and 28-d EC_{10} were generally above the expected application concentrations. *E. crypticus* and *F. candida* reproduction endpoints were often more sensitive to triclopyr when compared to imazapyr in the soils tested. Soil enzymatic activity could not be adequately modelled for dose response. However, for both the invertebrates and soil enzymes tested, clear site differences occurred in response to habitat quality specifically related to soil pH and total organic carbon.

Weight of Evidence (WOE) and Toxic Exposure Ratios (TER) were used to characterize the risks associated with herbicide application in northern latitudes providing both qualitative and quantitative means to effectively communicate the results to the public. In this study the WOE approach demonstrated that potential environmental concentrations were below not only the effective concentration at 25% (28-d EC_{25}), but also the effective concentration at 10% (28-d EC_{10}) values for all invertebrate species tested. While the TER approach identified that some ecological risk was present to soil organisms with the use of triclopyr but no unacceptable risks were identified through the application of imazapyr. The identified risks of triclopyr application are close to the critical trigger value of five and it is likely that soil invertebrate communities would recover less than one year after application.

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1. General Introduction

Vegetation management programs for transmission right-of-ways (ROWs) are essential to providing safe and reliable service to consumers. These vegetation management programs can integrate mechanical, chemical and biological techniques to ensure effective control of target vegetation. In northern climates, target vegetation is typically managed through mechanical techniques such as brushing and mowing. However, mechanical control can promote regrowth, especially for shrub and tree species that sucker (i.e. *Populus spp*. and *Salix spp*), increasing the density of target species and requiring more frequent management. There are over 1000 kilometres (km) of transmission lines in the Yukon Territory that need to be managed and incorporating herbicides into the vegetation management scheme may improve long-term effectiveness and decrease management costs. There is a data gap surrounding the fate and toxicity of herbicides in northern latitudes. Therefore, obtaining a greater understanding of the impact herbicides may have on northern terrestrial ecosystems is key to making informed vegetation management decisions. The aim of this project was to examine the environmental impact herbicides may have if added to vegetation management schemes in the Yukon Territory; specifically, the dissipation and toxicity of herbicides in soil.

1.1. Dissipation of Herbicides in the Soil

Degradation, persistence, residual concentrations, and dissipation are related to the residence time of herbicides in soil. Degradation is the breakdown of the herbicide molecule from the parent compound to metabolites by a chemical process (Mueller and Senseman, 2015). Soil processes contributing to the degradation of herbicides include adsorption, microbial degradation and photodegradation (Goetz et al., 1990; Baker and Mickelson, 1994; Johnson et al., 1995a; Gevao et al., 2000; Wang et al., 2006; Remucal, 2014). Persistence refers to the length of time herbicide residues are present in soil and bioavailable to organisms whereas residual concentration characterizes the actual herbicide present in the soil (Mueller and Senseman, 2015). Dissipation is considered the sum of all loss pathways of the parent compound (Mueller and Senseman, 2015). Various soil processes, properties and climatic conditions contribute to the dissipation of herbicide residues in the soil. Specifically, soil properties such as soil organic matter, clay content and pH influence dissipation rates, as well as, leaching and runoff (MacRae and Alexander, 1965; Goetz et al., 1990; Johnson et al., 1995a; Jourdan et al., 1998; Gevao et al., 2000; Wang et al., 2005a;

Douglass et al., 2016b). Climatic conditions including temperature and moisture will also influence dissipation pathways and thus attenuation rates.

1.1.1. Adsorption

Adsorption is the binding of ions or compounds to the outer soil surface and is considered a major contributor to the persistence of herbicide residues in soil (Goetz et al., 1990; Johnson et al., 1995a; Jourdan et al., 1998; Gevao et al., 2000; Dubus et al., 2001; Wang et al., 2006). Adsorption can occur via different mechanisms including: ionic, covalent and hydrogen bonding, electron donor and acceptors, Van der waals forces, ligand exchange, and hydrophilic bonding and partitioning (Gevao et al., 2000). The degree to which each of these mechanisms occur depends on soil texture, the herbicide's functional groups and the acidity of the system (Johnson et al., 1995a; Gevao et al., 2000; Dubus et al., 2001; Wang et al., 2006).

Soil texture regulates adsorption due to the characteristics of soil colloids; soil organic matter (SOM) and clay (Cantwell et al., 1989; Tilsworth et al., 1991; Johnson et al., 1995a; Gevao et al., 2000; Ashman and Puri, 2002). Soil colloids are small molecules that have large surface areas and electrostatic charges that result in greater adsorptive capacity (Jourdan et al., 1998; Ashman and Puri, 2002). Cation Exchange Capacity (CEC) regulates the degree to which a soil can exchange cations, which is measured by the amount of negatively charged exchange sites present on the soil colloids (Ashman and Puri, 2002). Due to the quantity of anionic exchange sites present, soils higher in SOM and clay at a neutral pH have higher CEC values. Higher CEC values indicate greater sorption potential resulting in longer residence times in soil when compared to soils with higher sand contents (Cantwell et al., 1989; Jourdan et al., 1998; Ashman and Puri, 2002). Acidic or anionic herbicides have higher leaching potentials since anions are not attracted to anionic colloidal surfaces and thus weakly sorb or do not adsorb at all (Ashman and Puri, 2002).

Herbicide molecules are composed of different functional groups that degrade at different rates, thus influencing adsorption (Goetz et al., 1990; Gevao et al., 2000). For instance, the herbicide molecule's functional groups can bind with phenolic and carboxylic groups that are found in humic material and are easily ionized (Gevao et al., 2000). Protonation of functional groups of acidic herbicides occurs at low pH values allowing for –COOH and –COOR groups to bind to the surface of the soil colloids. In these cases it is hydrogen bonding that allows the

herbicide molecules to bind to SOM but only at pH levels below their pka (Khan, 1973; Carringer et al., 1975; Senesei et al., 1984; Jourdan et al., 1998; Gevao et al., 2000; Dubus et al., 2001).

The acidity of the soil impacts the dissipation and movement of herbicides in the soil system by influencing the degree of adsorption to the soil colloids (Gevao et al., 2000). An inverse relationship exists between the soil pH and sorption where lower pH increases sorption leading to longer residence times due to less ionization of the herbicide particles (Tilsworth et al., 1991; Johnson et al., 1995a; Jourdan et al., 1998; Szmigielski et al., 2012; Gianelli et al., 2014). Further, Johnson et al. (1995) suggested that herbicides bind to weaker sites on the soil colloid ultimately affecting attenuation.

1.1.2. Microbial Degradation

A primary dissipation pathway for many herbicides is via microbial activity (MacRae and Alexander, 1965; Gevao et al., 2000; Kanissery and Sims, 2011; Douglass et al., 2016b). Specifically, microbial degradation involves two pathways: mineralization and co-metabolism (Felsot, 1989). Mineralization breaks the chemicals down to nutrients and energy that are bioavailable to plants and soil dwelling organisms (Felsot, 1989; Goetz et al., 1990; Ashman and Puri, 2002; Wang et al., 2006; Kanissery and Sims, 2011). In contrast, co-metabolism occurs when chemical molecules are degraded by soil enzymes (Felsot, 1989). For herbicides, the microbial community in the soil breaks down the least complex functional groups first before moving to the more complex groups, effectively breaking the parent herbicide into its metabolites (Ashman and Puri, 2002). The rates at which these degradation processes (mineralization and co-metabolism) occur depend on different climatic conditions (temperature, moisture content) and soil properties (SOM, pH) (MacRae and Alexander, 1965; Kanissery and Sims, 2011).

Microbial degradation is regulated by soil temperature and moisture content (MacRae and Alexander, 1965; Goetz et al., 1990; Jourdan et al., 1998; Barnes et al., 2009). Microbial degradation is temperature dependent where lower temperatures reduce degradation rates resulting in longer residence times in soil (Johnson et al., 1995a; Jourdan et al., 1998; Barnes et al., 2009). High microbial activity occurs in optimal moisture conditions, slowing considerably in drier, more acidic soils (Johnson et al., 1995a; Wang et al., 2005b). As moisture content increases, the bioactivity of degrading microbes increases. However, if the moisture content is beyond a threshold limit the bioactivity of the degraders will decline (Goetz et al., 1990). Favourable

precipitation is another major contributor to microbial degradation since it aids in the maintenance of soil moisture content and increases microbial respiration (Goetz et al., 1990). Precipitation is particularly important for sites with sandy soils and thin organic layers since it can be difficult for these soils to retain optimal moisture content for microbial communities (Goetz et al., 1990; Jourdan et al., 1998).

In addition to climatic conditions, SOM and pH can influence the microbial degradation of herbicides in soil. For instance, soils high in SOM and low pH affect the bioavailability of herbicide residues to microorganisms (Goetz et al., 1990; Jourdan et al., 1998; Wang et al., 2006; Barnes et al., 2009). Microbial degradation may be greater in soils with sandy texture due to lower sorptive capacity, which in turn increases potential bioavailability of the herbicide residues (Goetz et al., 1990; Jourdan et al., 1998). In addition, optimal microbial activity generally occurs in soils with neutral pH ranging from 6 – 7 (Jourdan et al., 1998). Lower bioavailability and bioactivity occur in soils with lower pH ranges due to increased adsorption capabilities.

1.1.3. Photochemical Degradation

In addition to adsorption and microbial degradation, photochemical degradation processes influence the dissipation of herbicides, accounting for up to 10% of the total residue degradation (Graebing et al., 2003; Orellana-Garcia et al., 2014; Remucal, 2014). Light within the natural light spectrum, including wavelengths greater than 290 nm, can photochemically degrade both active ingredients and formulations. Photochemical degradation can occur from both direct and indirect mechanisms (Falb et al., 1990; Konstantinou et al., 2001; Eyheraguibel et al., 2009; Remucal, 2014). In direct photolysis, ultraviolet (UV) light is absorbed by the herbicide molecule and is transformed via bond cleavage and the rearrangement of molecules to a more stable structure. Indirect photolysis occurs when light energy is absorbed by photosensitizing constituents within the media and produce reactive species that work to degrade the herbicide molecules (Torrents et al., 1997; Konstantinou et al., 2001; Remucal, 2014). In soil, photolysis occurs on the surface with dissipation rates depending heavily on soil properties and climatic conditions (Konstantinou et al., 2001).

The rate of photochemical degradation depends heavily on matrix composition, climatic conditions, and light intensity and penetration (Falb et al., 1990; Konstantinou et al., 2001; Eyheraguibel et al., 2009; Remucal, 2014). Organic matter has the greatest impact on dissipation

of herbicide residues in soil by aiding in indirect photolysis, by acting as a photosensitizer, or by acting as a source of hydroxyl radicals (McMartin et al., 2003). High iron oxides and hydroxyls within the soil matrix also impact degradation by creating a photo-Fenton reaction, accelerating degradation rates (McMartin et al., 2003). The quantity of daylight hours can also affect photochemical degradation by regulating the amount of UV light that reaches the soil surface, impacting both direct and indirect photolysis (Konstantinou et al., 2001; Graebing et al., 2003). Soil moisture content is important as it aids in diversity and abundance of soil micro-organisms which can degrade herbicide residues faster using the by-products of photolysis (Graebing et al., 2003).

1.1.4. Other Mechanisms of Herbicide Dissipation

As mentioned above, dissipation is defined as the sum of all herbicide loss pathways; therefore, it is important to consider all mechanisms when examining field dissipation of herbicides (Mueller and Senseman, 2015). Adsorption, microbial degradation, and photochemical degradation are the main processes associated with the dissipation of herbicides from soil, however, there are a few additional mechanisms that can also contribute to herbicide loss from soil. These mechanisms include volatilization, leaching, surface runoff, and uptake and metabolism by plants (Solomon et al., 1988; Goetz et al., 1990; Meru et al., 1990; Stephenson et al., 1990; Johnson et al., 1995a; Locke and Bryson, 1997; Barnes et al., 2009). Volatilization is regulated by Henry's law constant where a low constant indicates that herbicides are weakly volatile (Paszko et al., 2016). It also occurs more readily on vegetative surfaces and is limited once it hits the soil due to sorption and the lower temperatures of the soil surface. Sorption is the main limiting factor at the soil surface with lower evaporation potential and cooler temperatures also playing a role (Locke and Bryson, 1997). Ester-derived formulations volatilize more readily than acid formulations due to the higher vapour pressure of the esters (Barnes et al., 2009; Paszko et al., 2016). While triclopyr butoxy ester is used in many triclopyr based formulations, triclopyr hydrolyzes to the acid form so rapidly that minimal losses occur through volatilization (Barnes et al., 2009). Imidazoline herbicides, such as imazapyr, have a lower vapour pressure where less than 2% of the herbicide solution will volatilize. Therefore, volatilization is not likely an important dissipation pathway for these herbicides (Goetz et al., 1990).

High intensity precipitation events can cause vertical and lateral movement of the water-herbicide solution. Leaching and surface runoff are dissipation mechanisms that are of the greatest concern immediately after application prior to adsorption to the soil matrix (Meru et al., 1990; Stephenson et al., 1990; Locke and Bryson, 1997; Rice et al., 2007). Precipitation events immediately after application can lead to higher concentrations deeper in the soil column moving through the soil macropores (Sigua et al., 1995; Locke and Bryson, 1997). Significant surface runoff can occur in high precipitation events, however, residue concentrations in the runoff water are not present a couple weeks after application (Meru et al., 1990; Stephenson et al., 1990). High water solubility allows herbicide solutions to penetrate into the ground surface via reduced adsorption. However, most herbicide residues remain in the top 15 cm of the soil column (Locke and Bryson, 1997; Rice et al., 2007). Undisturbed soils are more heterogenous in nature which allows for greater movement through preferential flow pathways than disturbed soils (Tindall and Vencill, 1995; Locke and Bryson, 1997; Rice et al., 2007).

Vegetation interference is another mechanism that limits dissipation in soil, simply by the fact that herbicides do not reach the soil surface. In these cases, sorption and subsequent metabolism by the plants may transfer metabolites to the soil. However, delayed residual concentrations in the soil can occur when herbicide residues from decomposing plant material is transferred to the soil surface (Locke and Bryson, 1997). Within the plant-root zone dissipation is higher due to microbial activity with lower sorption capability (Mueller et al., 2014). Once microbial activity begins, the active ingredient is degraded into its metabolites at which point further dissipation occurs from the other mechanisms described above.

1.2. Ecological Risk Assessment and Toxicity Testing

Ecological risk assessment addresses and quantifies the environmental risk associated with a chemical or contaminant from an ecosystem perspective (Swartjes, 2011). There are two main types of ecological risk assessments: predictive and deterministic. Predictive methods are used to determine whether a chemical will have an impact on the ecosystems. These risk assessments generally include extrapolating laboratory data to real world situations to estimate potential risk to the ecological community (van Gestel, 2012). Deterministic risk assessments can be used to set application guidelines and clean-up criteria. This approach uses toxicity tests and bioassays and links them to environmental concentrations where unacceptable risk or uncertainty drives the

assessments to the next tier, which could include community level assessments (CCME, 1996; Jansch et al., 2006; van Gestel, 2012).

In Canada, there are a number of guidance documents on the use of ecological risk assessment including the Canadian Council for the Ministers of the Environment's (CCME) Framework for Ecological Risk Assessment, the Canadian Environmental Protection Act (CEPA) and the Federal Contaminated Sites Action Plan (FCSAP). These guidance documents use three components (problem formulations, hazard and exposure analysis, and risk characterization) to estimate use guidelines or clean-up criteria (CCME, 1996; Environment Canada, 1999, 2012). Problem formulation determines the scope and needs for the risk assessment, as well as develops a plan of how to assess those needs (CCME, 1996; Environment Canada, 2012). Problem formulation typically includes review of site or contaminant management objectives, review of existing data, and the determination of receptors and associated pathways (Environment Canada, 2012). Once the problem has been formulated, the exposure and hazard assessments can be initiated. The exposure assessment includes determining the actual or potential environmental concentrations, whereas, the hazard assessment typically involves reviewing published data and conducting dose response tests in a laboratory setting. Once the hazard analysis is completed associated risks can be estimated in the risk characterization step (CCME, 1996; Environment Canada, 2012; Swartjes, 2011).

Risk characterization summarizes the information obtained during the hazard analysis and uses it to develop a statement on the associated risks addressing the scope identified during problem formulation (CCME, 1996; Environment Canada, 1999, 2012). This can be done both qualitatively or quantitatively. Qualitative measures include Weight of Evidence (WOE) approaches that use the hazard analysis data coupled with professional expertise and judgement to characterize the risks (Environment Canada, 2007a). Quantitative approaches include the calculation of a specific trigger or action value, such as the use of toxic exposure ratios (TER). Quantitative approaches typically account for uncertainty thus providing a more conservative risk estimation. Further, quantitative approaches, such as TER and toxicological reference values, allow the risk assessor to easily determine whether higher tier assessment is required (Ernst et al., 2016).

From a soils perspective, soil ecotoxicology has lagged behind that of aquatic ecotoxicology due to the heterogeneous nature of soil and the difficulty associated with laboratory and field

testing (Environment Canada, 2007b). With the expansion of soil ecotoxicological studies, standardized single species toxicity test protocols have been developed to aid in the development of site-specific soil quality guidelines (van Gestel, 2012). These standardized toxicity tests include species with a range of sensitivities to ensure that chemicals are adequately characterized (Princz et al., 2012). Standardized tests also need to be ecologically relevant by incorporating species found at or near the sites being tested or at least representative of ecologically relevant species (Römbke et al., 2006b).

To achieve soil quality guidelines, the Canadian Council for the Ministers of the Environment (CCME) have developed ecological risk assessment procedures for developing environmental quality guidelines using a tiered approach. The tiered CCME approach involves three steps: screening assessment, preliminary quantitative assessment, and detailed quantitative assessment (CCME, 1996). Similar to pesticide risk assessments in the European Union, this is a deterministic approach where unacceptable risk or uncertainty drives the assessments to the next tier (CCME, 1996; Jansch et al., 2006). The screening assessment relies on published literature and data to make inferences about the level of risk a contaminant may play in the environment. The preliminary assessment addresses data gaps found in the screening level assessment using standardized procedures, such as laboratory toxicity tests. The final tier, the detailed quantitative assessment, gathers site specific data to draw conclusions about the risk a contaminant may impose on a system (CCME, 1996). The goal of this tiered approach is to ensure appropriate use guidelines and clean-up decisions are made that will maintain or recover ecological integrity (CCME, 1996).

While the use of terrestrial ecological risk assessments has been increasing in recent years, few studies have been published in the primary literature. Published studies from Canada tend to include the effect of contaminants on wildlife such as small mammals, birds and caribou and do not focus on soil dwelling fauna such as invertebrates and microorganisms (Braune et al., 1999; Gamberg et al., 2005). Soil dwelling organisms are needed to understand the role of contaminants on soil quality and overall ecosystem health. Single species testing can be used in conjunction with other single species tests to determine community level responses to the introduction of a contaminant (Römbke et al., 2006b; Princz et al., 2012). Toxicity assays can aid in the development of site-specific guidelines for herbicide usage which can help drive application rates.

1.2.1. Standardized Toxicity Testing

Standardized soil toxicity tests were lacking in Canada until the CCME published a framework for ecological risk assessment. Since publication the framework has become a fundamental component of ecological risk assessment for terrestrial sites in Canada (Römbke et al., 2006b; Environment Canada, 2007b). Recent research has focused on the role of soil dwelling organisms to determine applicability of specific invertebrate species for laboratory toxicity testing of contaminants in Canadian boreal and tundra soils (Römbke et al., 2006b; Princz et al., 2012). Conducting tests within a laboratory setting is important as it reduces variability within the results thus increasing confidence in those results (Stark et al., 1995; Moran, 1999; Princz et al., 2012). However, it should be noted that there is still some uncertainty when trying to apply the laboratory test results to field conditions (Moran, 1999; Princz et al., 2012).

Bioassays using soil invertebrates are important to gauge the toxicity of the bioavailable fraction of a given chemical, however, there is a lack of single species toxicity data for many chemicals (Loureiro et al., 2009). Further, the data available tends to focus on earthworms. A range of species with different sensitivities should be included in risk assessments (Frampton et al., 2006; Loureiro et al., 2009). Soil invertebrate species selected for laboratory toxicity assays are generally of ecological relevance and are easy to handle and maintain (Römbke et al., 2006b; Princz et al., 2012). Collembola and enchytraeid species are ideal because they are abundant and play an important role in the decomposition of organic matter and in the structure of soil (Jansch et al., 2006; Princz et al., 2010, 2012). Princz et al., (2010) determined that, due to their prevalence in boreal soil systems, Oribatid mites should be added to ecotoxicity testing in Canada. Therefore, to assess the effects of herbicides on terrestrial ecosystems along ROWs within the Yukon Territory three invertebrate groups (enchytraeids, collembola, mites) will be examined.

1.2.1.1. Enchytraeids

Enchytraeids, commonly known as pot worms, are found in the family Enchytraeidae (Oligochaeta, Annelida). Enchytraeids are small white worms that can reproduce asexually (Römbke, 2003). The genus, *Enchytraeus*, has been widely used in ecotoxicological testing due to their short generation cycles and high reproductive rates. *Enchytreaus crypticus* is unique in that it is often found on stressed or impacted sites (Römbke, 2003; Novais et al., 2010). In the Yukon Territory, enchytraeids are found in both brunisolic and cryosolic soil orders and are abundant in

layers rich in organic content where they feed on decomposing plant material and microorganisms (Smith et al., 1990; Didden, 1993; Römbke, 2003). The species, *E. crypticus*, resides in the upper soil horizons making it an ideal species for studying the impact of herbicides (Novais et al., 2010).

Enchytraeids have been shown to have chemical specific sensitivity with effects from herbicides strongly correlated to soil moisture. Enchytraeids thrive in high moisture soils and therefore exhibit lower survival and inhibited growth in drier conditions increasing the toxic effects of herbicides (Puurtinen and Martikainen, 1997). In fact, enchytraeids cannot live in conditions with less than 10% moisture content (Briones et al., 1997). Herbicides with high sorption capacity likely do not have adverse effects on enchytraeids since such herbicides have limited bioavailablity (Puurtinen and Martikainen, 1997). Herbicides such as phenmedipham and atrazine, have similar dose response patterns with adverse effects observed only at higher doses (Novais et al., 2010). In addition, low doses can cause a hormetic effect with higher reproduction rates at the expense of adult growth rates (Puurtinen and Martikainen, 1997; Arrate et al., 2002). Thus even at recommended application rates, the enchytraeid community could be altered (Didden and Römbke, 2001).

1.2.1.2. Collembola

Folsomia candida, or the compost springtail, is well studied, considered native to Canada, and is abundant in agricultural and forest soils (Fountain and Hopkin, 2005; Environment Canada, 2007b). The collembolan, *F. candida* has a preference for the fungal hyphae found on leaf litter. This species is a major contributor of decomposition and respiration within soil ecosystems (Fountain and Hopkin, 2005). In addition, collembola are an important prey species for soil predators including mites, beetles and centipedes (Hopkin, 1997; Fountain and Hopkin, 2005; Environment Canada, 2007b). The collembolan species, *F. candida,* is an ideal species for standardized testing as they are easily cultured under laboratory conditions and can live for up to 190 days (Environment Canada, 2014). Reproduction is rapid, occurring 12 to 16 days after hatching with eggs laid 5 to 7 days afterward. An individual can lay between 20 to 100 eggs. In ideal conditions eggs hatch within 7 to 10 days (Snider, 1973; Hopkin, 1997; Fountain and Hopkin, 2005; Environment Canada, 2014). Collembola can moult up to 45 times during their life span with the process occurring every three to eight days alternating between reproductive and non-reproductive instars (Fountain and Hopkin, 2005; Environment Canada, 2014).

The species, *F. candida,* has been found to be sensitive to pesticide exposure (Fountain and Hopkin, 2005; Daam et al., 2011), but few published papers investigating the toxic effects of herbicides on collembolan were found. A study using trisulfuron, a sulfonylurea herbicide with a mode of action similar to that of imazapyr, showed no adverse effects on survival or reproduction of the collembolan, *Onychiurus pseudogranulosus*, at up to six times the recommended field application rate (Sabatini et al., 1998). Further, this study found that the formulation had a more toxic effect than the active ingredient (Sabatini et al., 1998). High clay and SOM contents can act as buffers against toxic effects of phenmedipham to *F. candida*. However, the role soil properties play in the sensitivity of *F. candida* to herbicides is often too small to validate (Amorim et al., 2005a; Domene et al., 2011, 2012).

1.2.1.3. Oribatid Mites

Oribatid mites belong to the family Oppiidae, the largest family of mites. Mites in this family are challenging to use for toxicity testing due to their slow life cycles, but their role within the ecosystem make them a model candidate (Princz et al., 2010, 2012). Oribatid mites are important in terrestrial ecosystems due to their contribution to organic matter decomposition which directly aids in soil formation and nutrient cycling (Behan-Pelletier, 1997; Princz et al., 2010). These mites exist in most terrestrial ecosystems in the Yukon Territory and primarily feed on dead vegetation and fungi, as well as, lichens and carrion (Behan-Pelletier, 1997). Through their feeding habits and their external body structure these mites can also aid in the dispersal of bacteria and fungi (Behan-Pelletier, 1997). Since larger arthropods are often absent in northern ecosystems, presence of these mites is essential for nutrient cycling and soil formation (Behan, 1978; Behan-Pelletier, 1997).

Oppia nitens is an abundant mite species with the potential to inhabit many different soil types. However, this species prefers the upper horizon of forest soils with large amounts of fungi and organic material (Princz et al., 2010). Development from nymph to adult can range from 21 to 46 days depending on the temperature (Princz et al., 2010). In temperate climates adults live up to two years but individuals in northern climates can live for more than two years (Behan-Pelletier, 1997). No published studies on the effects of herbicides on Oribatid mites were found.

1.2.1.4. Enzymatic Activity as a Measure of Ecosystem Function

Upon exposure to chemicals and contaminants, non-target soil biota are often affected due to reduced primary producers, limited microbial diversity, and reduced soil fertility (Johnsen et al., 2001; Niemi et al., 2009). Herbicides limit soil nutrient cycles by various means including: altering soil composition and processes, stimulating bioactivity, and/or increasing enzyme excretion (Niemi et al., 2009). Herbicide application and its effects on soil enzyme activity are typically dose dependent and can either stimulate or inhibit enzymatic activity (Niemi et al., 2009; Floch et al., 2011). Furthermore, herbicide formulations are important to use for these tests as the formulation is often more toxic to microorganisms than the active ingredient alone (Niemi et al., 2009).

Herbicides can have a negative impact on the function of soil enzymes and changes in enzyme activity levels can provide early indications of impacted nutrient cycling when compared with other dissipation parameters, such as soil properties (Floch et al., 2011). As the cell structure of microorganisms is disrupted a measurable shift in enzyme activity can occur (Floch et al., 2011). Herbicides alter enzyme activity in the soil by binding with the active proteins of the enzyme molecule resulting in stimulation or inhibition (Tabatabai, 1994; Floch et al., 2011). Assessment of the effect of herbicides can be broken down into different classes of microorganisms, including nitrogen, phosphorus, and carbon reducing bacteria that aid in the cycling of nutrients within the soil ecosystem (Ashman and Puri, 2002). Using soil enzyme assays, conclusions can be drawn about the effect of herbicides on soil microbial communities and the associated nutrient cycling processes (Felsot, 1989).

1.3. Vegetation Management and Herbicide Use

To ensure consistent service to consumers and reduce risks associated along transmission right-of-ways (ROWs) vegetation management must be conducted. Good management programs are an important tool that require knowledge of numerous subject areas including soil and herbicide properties, vegetation communities, overall management objectives, and social license. In the early 1900's, mechanical control techniques were the first to be employed (Brown, 1995). Herbicide use was adopted in the 1940's and due to its efficacy, rapidly became the norm for vegetation management along transmission ROWs (Brown, 1995; Sulak and Kielbaso, 2000). In the 1950's, increased public awareness and research of the harmful effects of herbicides to both the environment and human health led to the development of integrated vegetation management

practices (Geier et al., 1992; Nowak and Ballard, 2005a). Integrated Vegetation Management (IVM) practices incorporate different tools that work to effectively control target species while also reducing the risk to non-target species and minimizing soil disturbance (Nowak and Ballard, 2005a; Yahner, 2006; Thiffault and Roy, 2011; Douglass et al., 2016b). As such, many different control measures are incorporated into an IVM program. In addition to control techniques, IVM programs must utilize information on vegetation community change, dissipation of herbicides in soil and vegetation, toxicological concerns, and effective communication strategies (Nowak and Ballard, 2005b; McLoughlin, 2014). Incorporating all these aspects will allow for an effective and adaptable management strategy for ROW managers.

1.3.1. Herbicides for Use Along ROWs in the Yukon Territory

In 2012, Environmental Dynamics Inc. (EDI) was contracted to conduct a review on the feasibility of incorporating herbicide treatments into Yukon Energy Corporation's (YEC) existing vegetation management program. Fourteen herbicides were ranked based on effective management of the target species, as well as, risk to the environment and human health. The review identified four active ingredients (aminopyralid, glyphosate, triclopyr and imazapyr) in commonly used commercial herbicide formulations which could be effective along ROWs within the Yukon (EDI, 2013). A field trial with glyphosate, triclopyr and imazapyr was conducted by EDI in the summer of 2013. The results indicated that triclopyr and imazapyr would be good candidates for further investigation along ROWs in the Yukon Territory (EDI, 2013).

1.3.1.1. Triclopyr

Triclopyr or 3,5,6-trichloro-2-pyridinyloxyacetic acid, is a pyridine equivalent of phenoxy herbicides used on industrial sites and along ROWs to selectively control broadleaf weeds and woody species (Solomon et al., 1988; Johnson et al., 1995a). Triclopyr is absorbed readily by foliage and translocated rapidly throughout the apoplastic and symplastic systems (Pitt et al., 1993; Senseman, 2007; Barnes et al., 2009; Grossmann, 2009). Auxin hormones influence many growth and development processes in plants. Synthetic auxin herbicides, like triclopyr, mimic these growth hormones allowing the plant to grow without regulation until it grows itself to death (Cobb, 1992; Sterling and Hall, 1997; Fedtke and Duke, 2005; Senseman, 2007; Grossmann, 2009).

Triclopyr is produced in both acid and ester formulations with the acid being more water soluble and the ester being an oil soluble formulation (Barnes et al., 2009). Garlon XRT, used in this project, is formulated with triclopyr butoxyethyl ester (TBE). Triclopyr butoxy ester itself has a half-life of 1.1 days but is rapidly hydrolyzed to the acid form in soil resulting in an average half-life of 32 days (Barnes et al., 2009). Triclopyr is a weakly acidic herbicide with a pKa of 2.6 (Johnson et al., 1995a). At standard field pH the acid will deprotonate resulting in a negative charge that will cause the herbicide to bind weakly to soil colloids. In acidic soils (pH<5), the herbicide, will be present in less polar forms allowing it to sorb more readily to the soil increasing residence time (Johnson et al., 1995a).

1.3.1.2. Imazapyr

Imazapyr, [2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid], belonging to the imidazolinone family, is a non-selective herbicide used for control of grasses and broadleaf plants (Wang et al., 2005b; Ramezani et al., 2010; Gianelli et al., 2014). Imazapyr absorbs rapidly into plant tissue, mainly foliage and roots (Pusino et al., 1997). Imazapyr inhibits acetolactate synthase (ALS), an enzyme responsible for the synthesis of branched chain amino acids (valine, leucine and isoleucine) in plants. Once at the site of action the herbicide inhibits ALS causing meristematic tissue injury which constrains plant growth ultimately resulting in plant death (Stidham, 1991; Masson and Webster, 2001; Heiser, 2007).

Imazapyr is a weakly acidic herbicide, with pKa range of 1.9 to 3.6, that adsorbs weakly to soil (Pusino et al., 1997). It has a high solubility indicating a greater potential for leaching than that of triclopyr (Wang et al., 2006). Microbial degradation is the primary dissipation pathway accounting for up to 78% of the disappearance of imazapyr in non-sterile soils (Wang et al., 2006). However, there is high variability in the half-lives and dissipation trends among aerobic and anaerobic conditions (Wang et al., 2006). A neutral pH with optimal moisture levels are more favourable for degradation since it provides optimal conditions for microbial degradation (Wang et al., 2005a; Douglass et al., 2016b). Wang et al. (2005a) showed that as pH increased, imazapyr and its metabolites persisted longer in soils. In addition, they were able to show that soils with high organic matter content had increased degradation rates due to increased microbial rates (Wang et al., 2005a).

1.3.2. Application Techniques for Use in the Yukon Territory

Different application techniques can be utilized in IVM programs to ensure efficient and cost-effective management of ROWs. Mechanical and chemical practices are the predominant application techniques used today, however, biological practices, including selective removal and seeding, are becoming more prevalent with increased public awareness of the effects that herbicides can have on the ecosystem. Mechanical control is currently used as the sole management technique in the Yukon Territory. This technique provides immediate control of target species through mowing, brushing or manual tree removal. Mowing consists of a tractor mounted with a cutting adapter that is capable of removing a 2.5 m wide strip of vegetation (Annighofer et al., 2012; EDI, 2013). The cutting adapter cuts into the soil degrading the surface thereby destroying soil structure and increasing the chance of soil erosion. Management plans that depend solely on mechanical control techniques are limited in that the woody deciduous target species regrow rapidly via stump and root sprouts (Niering and Goodwin, 1974; Bramble et al., 1991; Berkowitz et al., 1995; Illisson and Chen, 2009; Thiffault and Roy, 2011).

Herbicide use in vegetation management plans has proven to be effective against target species long-term (Niering and Goodwin, 1974; Bramble et al., 1991). The three herbicide application methods examined in this study included: foliar spray, cut stump and point injection. Foliar spray can be applied at both high and low volume application rates. High volume foliar spray or broadcast application involves a boom with a series of fixed nozzles attached to a truck or ATV (Nowak and Ballard, 2005a; Barnes et al., 2009). Low volume foliar spray is applied using backpack sprayers (Nowak and Ballard, 2005a). Cut stump involves removing a tree at ground surface and applying herbicide directly to the stump, focusing on the cambium (Ballard and Nowak, 2006). Point injection, the final control measure, involves cutting the tree surface to the cambium and injecting a small volume of concentrated herbicide mix.

Biological control techniques, also known as ecological manipulation, involve altering the ecosystem allowing non-target vegetation species to flourish while eliminating target species (de Blois et al., 2004). One way to perform this technique is through selective cutting of target species followed by seeding of native grass or shrub species. The combination of selective removal and seeding is designed to promote a low growing vegetation community, ultimately reducing management cycles (de Blois et al., 2004).

1.3.3. Herbicide Attenuation in Northern Soils

Transmission ROWs in the Yukon Territory are typically found on soils classified as eutric brunisols. The brunisolic order consists of poorly developed soils with limited illuvial clay deposits and few aluminum and iron complexes (Smith et al., 2011). These soils are typical in montane, mixed wood and boreal environments. Brunisols are also often located adjacent to cryosolic soils (Soil Classification Working Group, 1998; Smith et al., 2011). Specifically, eutric brunisols have thin organic surface layers and a brownish B horizon that has been altered by hydrolysis, oxidation and/or solution with a pH greater than 5.5 in at least the top 25 cm of the B horizon (Soil Classification Working Group, 1998; Smith et al., 2011). Eutric brunisols tend to be found on alkaline parent materials in coniferous or mixed wood forests (Smith et al., 2011). These soils are typically found at low elevations and in areas with low seasonal temperatures and minimal precipitation (< 350 mm) (Smith et al., 2011). Recognizing differences in soil types along ROWs in the Yukon Territory is important because it will directly impact the degradation pathways applicable to each herbicide.

Adsorption capacity is likely low in the Yukon Territory as the eutric brunisols that dominate the region have thin organic layers and minimal amounts of clay (Jourdan et al., 1998; Graebing et al., 2003; Smith et al., 2011). These sandy soils may also be more prone to leaching as there are fewer colloidal surfaces for sorption of the herbicides. Temperature is a primary driver of residue degradation in northern latitudes. Throughout the year there are extreme temperature changes with only four to five months where the soil is frost free (Wahl, 2004). Newton et al. (2008) noted that dissipation patterns vary with season, increasing in the summer when conditions are favourable, but slow during the winter. Dissipation rates slow in the winter due to reduced microbial activity when temperatures fall below freezing (Newton et al., 2008; Barnes et al., 2009). Due to approximately 19 hours of direct sunlight during prime application season, photochemical degradation is key in the dissipation of herbicide residues in the North. Soil composition and climatic factors in northern ecosystems need to be considered to fully understand the dissipation of herbicides at northern latitudes.

While no published dissipation studies from Canada's Territories were found, previous studies indicated that triclopyr and imazapyr residues were present at least two years after application in Alaskan soils (Newton et al., 2008; Barnes et al., 2009). These studies also indicated

that soil samples were only collected for two years after application and it is unknown how long the residual concentrations were present (Newton et al., 2008; Barnes et al., 2009). In the boreal region of Sweden, triclopyr residues were observed two years after application with dissipation rates slower in northern latitudes (Torstenssen and Stark, 1982). Stephenson et al. (1990) investigated the dissipation of triclopyr in Northern Ontario and found that although residues were present for long periods, concentrations were below 10% of the application rate after 28 days, which is likely below a concentration that presents a significant risk to human or ecological health. Along railway embankments in Sweden, imazapyr had half-lives that ranged from 67 to 144 days after treatment (Börjesson et al., 2004). The different application techniques discussed above directly influence the presence of herbicides in soil. For instance, foliar spray application is less targeted than cut stump and point injection applications and is more likely to contact the ground surface during application. Cut stump and point injection treatments are applied directly to the stem and, as a result, concentrations in the soil may not be present immediately. However, herbicide residues in foliage may be transferred to the soil from treated vegetation resulting in delayed soil contact and hypothetically a longer dissipation time (Thompson et al., 1994).

Mechanical techniques, including brushing and mowing, are currently the only control methods used on transmission ROWs in the Yukon Territory. Mechanical practices are not without problems including short maintenance cycles due to increased regrowth and density of target species, destruction of wildlife habitat, and reduced vegetative cover increasing susceptibility of soil erosion (Nickerson, 1992; EDI, 2013). Mechanical methods are also typically more expensive than other methods based on labour, equipment and fuel consumption (BASF, 2005; Johnson, 2008). Long-term vegetation control along transmission right-of-ways (ROWs) in the Yukon Territory may be improved through incorporating different management practices. These practices could integrate mechanical and chemical vegetation management with regional or site-specific techniques, such as selective removal and seeding. Specifically, incorporating herbicides into the vegetation management program could improve long-term effectiveness, reduce environmental risks, and decrease management costs.

Due to the unique soil chemistry, cold climates and short growing seasons in the Yukon, data gaps surrounding the dissipation and associated toxicity of these herbicides in northern terrestrial ecosystems was investigated. Determining ecosystem response of adding herbicides to

vegetation management regimes depends on numerous factors including species abundance and diversity prior to application, environmental conditions at the site of application and herbicide selection. Understanding site-specific factors that contribute to toxicity of the herbicides is key to making informed management decisions (Princz et al., 2012).

1.3.4. Effects of Triclopyr and Imazapyr on Non-target Species

While no information pertaining to the species listed in section 1.2.1 were located, there is published literature surrounding the toxicity of triclopyr and imazapyr to other non-target wildlife. The effects of triclopyr to stream invertebrates found that the toxicity estimates were a 1000-fold higher than the expected potential environmental concentrations. Additionally, while triclopyr residues accumulated in submerged leaf litter, no toxic effects were observed even at the highest concentrations (Peterson et al., 2001). The triclopyr ester form is known to be highly toxic to fish and somewhat toxic to aquatic invertebrates. However, the susceptible period of exposure to the ester form is short because the ester form hydrolyzes to the acid form within one day (Tatum, 2004; Barnes et al., 2009). Further, studies examining the indirect effects of triclopyr applications have identified no impacts on the diversity of arthropod communities (Bramble et al., 1999; Fuhlendorf et al., 2002). Imazapyr, on the other hand, is considered to be essentially nontoxic with a LD_{50} greater than 2000 mg a.i. kg body weight^{-1} (Tatum, 2004). A study examining the effect of imazapyr, applied as a tank mix, to the Oregon spotted frog (*Ranas pretiosa*) at an application rate of 7.0 L ha^{-1} posed no unacceptable risk (Yahnke et al., 2013). Further, effects associated with the application of imazapyr indicated no significant changes to morphology, composition or biomass to benthic invertebrates when applied at normal application rates in a shallow basin in Florida (Fowlkes et al., 2003; NCASI., 2004). Overall, triclopyr and imazapyr are considered to present no unacceptable risks to non-target invertebrate and wildlife species since they degrade quickly and do not bioaccumulate (NCASI., 2003, 2004; Tatum, 2004).

Herbicides, such as triclopyr and imazapyr, are specifically formulated to control target vegetation, however, due to their specific mode of actions impacts on non-target vegetation are anticipated. It is important to quantify the impact of chemical control on non-target vegetation so application rates can be adjusted to ensure that recovery of non-target species is possible. Isbister (2016) identified that non-target Yukon species recovery was lifeform specific. Non-target vascular species had small population decreases one year after application with triclopyr (Isbister,

2016). Based on published studies, it is likely that the herbaceous community will fully recover two years after application while shrubs may take up to five years to recover after triclopyr application (Sullivan et al., 1996; Newmaster and Bell, 2002; Man et al., 2010; Seefeldt et al., 2013; Isbister, 2016). The effects of imazapyr on non-target species was more pronounced than triclopyr. Germination was still possible in treated areas but with visible deformities (Isbister, 2016). A foliar application study on *Chamerion angustifolium*, a non-target species found along Yukon Territory ROWs, identified similar trends. Results from a study of foliar applied triclopyr identified the concentration that would inhibit 50% germination (28-d IC_{50}) to be less than 50% of the maximum application rate, whereas, the 28-d IC_{50} for imazapyr was less than 2% (Isbister et al., 2017). Therefore, it is important to understand the sensitivities of target species prior to herbicide application to ensure that appropriate application rates are used to allow for recovery of non-target communities.

1.3.5. Objectives and Hypotheses

The overall objective of this research project was to evaluate environmental risk associated with adding herbicides to Yukon Energy Corporation's (YEC) vegetation management strategy for ROWs. By assessing the toxicity and dissipation of Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr), the ecological risk to northern soils when applied at recommended application rates was characterized for transmission ROWs in the Yukon Territory. Specifically, it was hypothesized that herbicide residues will be present in soils longer than 365 days along Yukon ROWs. However, these residues will be below a concentration that adversely affects more than 25% of the soil community when sprayed at or below recommended field application rates. Formally, these hypotheses can be stated as:

- H_{01}: Herbicide residues will be present in Yukon Territory soils for less than 365 days.
    - H_{a1}: Herbicide residues will be present in Yukon Territory soils for greater than 365 days.
- H_{02}: Herbicide concentrations in soil will impact less than 25% of the soil ecological community.
    - H_{a2}: Herbicide concentrations in soil will impact greater than 25% of the soil ecological community.

As suggested by the hypotheses, the project was divided into two main components: field dissipation and laboratory toxicity. Chapter 2 outlines the field dissipation study that examines

dissipation patterns in soil at a site representative of Yukon Territory ROWs, as well as, compares herbicide concentrations at four other sites. Chapter 3 describes the toxicity of triclopyr and imazapyr to three soil organisms and three soil enzymes present in soils representative of Yukon Territory ROWs. The concentrations of herbicide residues that can negatively impact the soil ecological community was determined. These chapters are followed by a synthesis chapter (Chapter 4) that links data from the dissipation and toxicity studies to evaluate the risks associated with adding herbicides to the vegetation management strategy in the Yukon Territory.

PREFACE: CHAPTER 2

Shortened vegetation management cycles along transmission ROWs in the Yukon Territory are the result of mechanical control techniques. Thus, alternative control measures, including herbicide application, were examined. Herbicide use in the Yukon Territory is limited and, as such, there is a data gap surrounding the fate of these chemicals in this region. Assessing the dissipation patterns of triclopyr and imazapyr in soils representative of Yukon Territory soils is an important first step in the evaluating the risk these herbicides may pose to soil ecosystems along transmission ROWs. The first data chapter examines the dissipation patterns of triclopyr and imazapyr in soils representative of Yukon Territory ROWs. The dissipation rate for each herbicide was assessed from one site where soils were collected at numerous intervals after application. Soils were collected at an additional four sites to compare residue concentrations at three defined intervals. Knowledge of dissipation rates and patterns is critical for implementation of herbicide use in the North. The dissipation results will be used in conjunction with the toxicity data presented in Chapter 3 to estimate the risk associated with adding herbicides to the vegetation management scheme along ROWs in the Yukon Territory.

2. DISSIPATION OF TRICLOPYR AND IMAZAPYR IN SOIL ALONG TRANSMISSION RIGHT-OF-WAYS IN THE YUKON TERRITORY

2.1. INTRODUCTION

Control of fast growing woody vegetation along transmission right-of-ways (ROW) is essential for ensuring safe and reliable electrical service. Vegetation management in northern environments is challenging and costly. Integrated vegetation management (IVM) may provide an approach to effectively control vegetation on ROWs through adaptive control techniques. Herbicide treatment is an effective control technique in temperate regions, but has not been widely applied in northern climates, resulting in a lack of understanding of the fate of these compounds in cold environments (Torstenssen and Stark, 1982; Börjesson et al., 2004; Newton et al., 2008; Barnes et al., 2009; Douglass et al., 2016). Determining the dissipation patterns of herbicides in soil and vegetation from a northern ecosystem perspective is important to the development of appropriate IVM programs. Based on the results of a 2014 pilot study, Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr), common forest herbicides used along right-of-ways, were selected as the best candidates to examine the impacts of herbicide use in the Yukon Territory (EDI, 2013). Here the dissipation of triclopyr and imazapyr in soils along ROWs in the Yukon Territory, Canada was assessed.

In northern climates, dissipation of triclopyr and imazapyr from soils occurs primarily during the growing season, slowing noticeably in the winter (Newton et al., 2008). In Alaska, detectable triclopyr residues were present two years after treatment (Mulkey, 1990; Newton et al., 2008; Barnes et al., 2009). However, since soil samples were only collected for two years after application it is unknown how long the residues are present (Newton et al., 2008; Barnes et al., 2009). Detectable residues of triclopyr in the boreal forest region of Sweden were observed over two years after treatment with lower dissipation rates consistently observed in northernmost sites (Torstenssen and Stark, 1982). Stephenson et al. (1990) investigated the persistence of triclopyr in Northern Ontario and found that although residues were present for long periods, concentrations were below 10% of the application rate within 28 days after treatment, which is likely below a concentration that presents a risk to human or ecological health. Imazapyr is known to have long residence in soils (Senseman, 2007; Douglass et al., 2016b) and was detected 456 days after application in Alaska (Newton et al., 2008). Along railway embankments in Sweden, imazapyr

half-lives ranged from 67 to 144 days after treatment with residues detected in the groundwater eight years after application (Börjesson et al., 2004). Dissipation mechanisms were not examined in detail, however, it is hypothesized that the dissipation patterns observed were primarily due to microbial and photochemical processes linked to soil characteristics such as pH, texture, organic matter content, temperature and moisture content (Torstenssen and Stark, 1982; Stephenson et al., 1990; Johnson et al., 1995a; Borjesson et al., 2004; Wang et al., 2005b; Newton et al., 2008; Barnes et al., 2009).

The primary dissipation pathways for triclopyr and imazapyr in soil are microbial and photochemical degradation. These pathways are influenced by climatic conditions and soil properties. Ideal soil temperature and moisture content aid in the diversity and abundance of microorganisms that act on the herbicide residues. In addition, these microorganisms can act more readily on the by-products of photolysis, decreasing soil residence time (Graebing et al., 2003). Acidic herbicides, such as triclopyr and imazapyr, tend to sorb weakly to soils making them less persistent due to greater availability to microorganisms (Gianelli et al., 2014). Although acidic herbicides sorb weakly, soil properties including pH, clay content and soil organic matter can alter the degree to which the herbicides adsorb to soil particles (Johnson et al., 1995a; Gianelli et al., 2014; Douglass et al., 2016b). Soils with pH levels below 5 have stronger sorption capacities slowing dissipation rates due to less ionization of the herbicide molecules (Johnson et al., 1995a; Jourdan et al., 1998; Szmigielski et al., 2012; Gianelli et al., 2014). Herbicide residues in soils with high clay and organic matter contents dissipate at slower rates due to increased sorption and less availability for chemical and biological degradation pathways (Stephenson et al., 1990; Pusino et al., 1997; Szmigielski et al., 2009; Gianelli et al., 2014). Differences in climate and soil composition play an important role in the dissipation patterns of herbicides. Therefore, it is important to study dissipation rates and patterns in the North where cold climatic conditions and poorly developed soils exist.

Vegetation management along ROWs in the Yukon Territory employs mechanical control techniques only, including brushing and mowing. Brushing and mowing result in increased density and regrowth of target species including *Populus spp.* and *Salix spp.* (Nickerson, 1992; Nowak and Ballard, 2005a). Long-term vegetation control along transmission ROWs in the Yukon Territory may be improved through incorporating different management practices. These practices

could integrate mechanical and chemical vegetation management with regional or site-specific techniques, such as the seeding of desirable species. Incorporating herbicides into the vegetation management program could decrease management costs, improve long-term effectiveness and reduce environmental impacts.

The purpose of this study was to evaluate the spatial dissipation patterns of triclopyr and imazapyr along northern boreal ROWs. Specifically, the objective was to determine the dissipation rates of both triclopyr and imazapyr from soil at a site representative of Yukon Territory ROWs, as well as, to compare dissipation rates at four additional sites to determine if site-specific differences are present. Obtaining a better understanding of the fate of herbicides in northern climates will allow managers to evaluate the risk associated with adding herbicide application to the existing management regime. Ultimately, this will aid in the development of appropriate management solutions for transmission ROWs in the Yukon Territory and other northern settings.

2.2. METHODS

2.2.1. Site Locations

Five sites (CAR, DAW, HJ1, HJ2, LS) were selected along transmission ROWs in the Yukon Territory, Canada, to assess the dissipation of two herbicides, triclopyr and imazapyr, in northern soils. Specific site locations were selected based on vegetative communities that were representative of the area and of an appropriate age to apply treatments (Table 2.1). Soils at each site are classified as eutric brunisols with a silt loam texture. Table 2.2 summarizes specific soil properties for each site.

Table 2.1. Summary of site information including GPS co-ordinates, ecoregion, mean annual precipitation, mean January and July temperatures, last mowing cycle, treatment application date and precipitation 2 days after treatment (DAT). Modified from Isbister, 2016.

Site	Abbrev. †	Coordinates	Ecoregion‡	Last Mowing Cycle	Mean Annual Precipitation§	Mean January Temp§	Mean July Temp§	Treatment Application	Precipitation 2 DAT#
					(mm)	(°C)	(°C)	(dd-mmm-yy)	(mm)
Carmacks¶	CAR	61.8° N, 136.0° W, 61.9° N, 136.1° W	Yukon Plateau - Central	2010	323.4	-17.2	14.9	15-Jul-14	0.0
Dawson	DAW	63.9° N, 138.4° W	Yukon Plateau - North/ Klondike Plateau	2008	324.3	-26.0	15.7	3-Aug-14	1.1
Haines Junction 1	HJ1	60.8° N, 136.6° W	Yukon Southern Lakes	2013	297.3	-16.1	13.0	19-Jul14	0.0
Haines Junction 2	HJ2	60.8° N, 136.0° W	Yukon Southern Lakes	2011	297.3	-16.1	13.0	25-Jul-14	0.0
Little Salmon	LS	62.1° N, 135.1° W	Yukon Plateau - Central	2014	319.7	-21.1	14.0	28-Jun-15	9.6*

† Abbrev.: Abbreviation. ‡ As per 'Ecoregions of the Yukon Territory: Biophysical properties of Yukon landscapes' (Smith et al., 2004). § Weather data obtained from Environment Canada 1981-2010 Climate Normals: Mayo Road (CAR), Dawson Airport (DAW), Otter Falls (HJ1 & HJ2) and Drury Creek (LS). ¶ Carmacks site was divided into two sections due to the variability of vegetation types on the right-of-way. Blocks 1 & 2 were installed on 15-Jul-14 while Block 3 was installed on 21-Jul-14. # Precipitation data obtained from Historical Weather Data (Environment Canada, 2015). * Precipitation data obtained from the Faro weather station as no 2015 information was available from the Drury Creek station.

Table 2.2. Mean and standard error (SE) of selected soil properties (mean ± SE, n=3) used for the study of triclopyr and imazapyr dissipation of five sites in the Yukon Territory, Canada.

Site	Moisture	pH	Total Organic Carbon	Clay	Silt	Sand
	(%)	(unitless)	(%)	(%)	(%)	(%)
CAR	8.0 ± 0.11	6.1 ± 0.16	16 ± 1.1	13 ± 1.0	9.2 ± 2.5	51 ± 2.3
DAW	12 ± 0.81	4.7 ± 0.09	12 ± 0.65	16 ± 0.73	44 ± 1.8	19 ± 1.0
HJ1	13 ± 1.4	6.2 ± 0.07	16 ± 1.9	11 ± 0.94	18 ± 5.5	51 ± 4.3
HJ2	13 ± 2.7	7.0 ± 0.05	17 ± 3.4	12 ± 1.1	38 ± 2.5	31 ± 3.1
LS	1.8 ± 0.06	5.4 ± 0.06	4.2 ± 0.41	4.7 ± 0.14	53 ± 2.0	37 ± 2.2

2.2.2. Study Design

A completely randomized block design including three blocks consisting of eight 6 m^2 treatment plots were installed at each site (Figure 2.1). Each 6 m^2 treatment plot was placed in the centre of a 30 m wide ROW and was separated by a minimum of 50 m to eliminate the risk of influencing adjacent plots. Two herbicides commonly used along ROWs 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 isopropylamine salt; BASF Canada Inc., Mississauga, ON). Treatment application dates are included in Table 2.1.

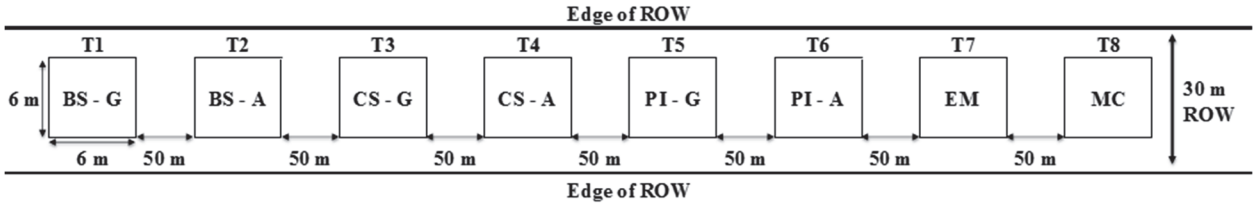

Figure 2.1. Study design for Yukon Energy transmission ROWs examining herbicide dissipation in soils. Example of a block within our complete randomized block design with 8 treatments: backpack spraying (BS), cut stump (CS), point injection (PI) with 2 herbicides (Garlon XRT (G) and Arsenal Powerline (A)) and ecological manipulation (EM) and a mowing only control (MC). Three blocks were established at each site for a total of 120 treatment plots.

Eight vegetation management treatments including three herbicide application techniques using each herbicide (backpack spraying, cut stump and point injection); seeding and selective harvest and mowing (control) were implemented at each site. For the backpack spraying treatment, herbicides were applied at the highest manufacturer recommended rate with a Stanley 61804 Poly 4 Gallon Professional Backpack Sprayer. Application rates were 4.5 kilograms of active ingredient per hectare (kg a.i. ha^{-1}) and 0.72 kg a.i. ha^{-1} for triclopyr and imazapyr, respectively. Tree species higher than 1.5 m were cut close to ground surface (~25 cm) prior to spraying with cut sections removed from the plot area. In the cut stump treatment, all vegetation was cut at 20-30 cm above ground surface and herbicides were applied to the cut surfaces with a paint brush. Maximum rates specified on the manufacturers label were used for the cut stump application. Solutions consisting of 19% Garlon XRT (143.5 g triclopyr L^{-1} canola oil) and 9.4% Arsenal® Powerline (22.6 g imazapyr L^{-1} DI water), respectively, were applied. The point injection treatment consisted of cutting target species to the cambium and injecting a small volume of herbicide with a 20 mL

syringe. Solution concentrations were the same as for the cut stump treatment. Hasten™ Spray Adjuvant (704g L^{-1} Ethyl and Methyl esters of vegetable oil with 196 g L^{-1} non-ionic surfactants; Victorian Chemicals Group, Victoria, AUS) was used for all Arsenal Powerline treatments and was added to application solutions at rate of 0.25%. For the seeding treatment, target species were removed approximately 10-20 cm above ground surface using pruners and/or a handsaw. The point injection and seeding plots were raked and seeded with the following mix of native grass species: 42% *Elymus violaceum*, 36% *Elymus trachycaulus*, 8% *Festuca saximontana*, 6% *Poa glauca*, 5% *Calamagrostis canadensis* and 2% *Deschampsia caespitosa*. Seed was obtained from DLF Pickseed Canada (Lindsay, ON) and was hand broadcast at a rate of 50 kg ha^{-1} (Isbister, 2016). Mowing was used as a control to represent existing management techniques and involved the removal of all vegetation 10-30 cm above ground surface.

2.2.3. Soil Sampling

The upper soil horizon (0-3 cm) consisting primarily of organic soil was sampled. A trowel with a depth gauge was used to sample areas approximately 8 cm in diameter to a depth of 3 cm (upper soil layer) regardless of the depth of the organic layer (Figure 2.2). Organic matter thickness was recorded at each sample location and varied between plots ranging from < 0.5 cm to 16 cm. Therefore, amounts of organic matter in the upper soil layers varied between samples, but was representative of the upper horizontal characteristics of each site. Soil samples were randomly collected at three locations within the treatment plot to ensure a representative sample from within the treatment area (Figure 2.2). Upper soil horizon samples, each approximately 167 cm^3 were tightly packed into a single 500 mL Nalgene, (~500 cm^3) bottle. Reference samples were collected from all plots prior to treatment following the same procedure.

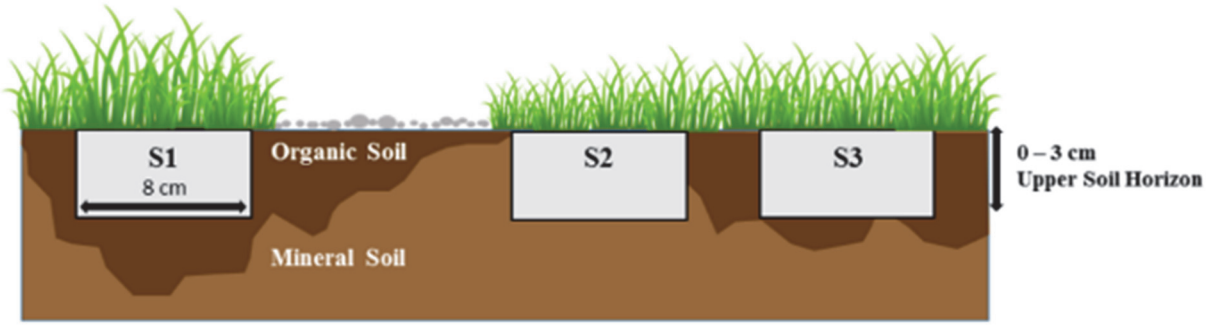

Figure 2.2. Three samples with a cumulative volume of 500 cm^3 were collected from the upper soil horizon (0-3 cm). Samples within treatment plots were collected at three locations (S1-S3) to capture variability in organic matter depth. As shown, organic matter thickness is variable within plots resulting in the 0-3 cm sample comprised of both organic and mineral soil.

Treated soils were collected from each plot at 1, 30 and 365 days after treatment (DAT). At the LS site, soil samples were collected as soon as herbicides had dried (~ 2 hours after application) and at 1, 3, 7, 14, 21, 30 and 60 DAT. Increased sampling intervals were used at LS to better determine the dissipation rate of each herbicide in a soil representative of Yukon Territory ROWs.

After each sample collection, the trowel was decontaminated by removing excess soil with a scrub brush and then rinsed with acetone followed by de-ionized water. The trowel was then dried with clean paper towels and placed into a clean Ziploc bag. Samples were stored at a temperature of approximately -5 ^oC until analysis. Prior to analysis samples were air dried in a dark room and sieved to 2 mm. Samples were shipped to the University of Guelph's Agriculture and Food Laboratory for herbicide residue analysis.

2.2.4. Chemical Analysis

Samples were analyzed at the University of Guelph's Food and Agriculture Laboratory using High Performance Liquid Chromatography coupled with tandem Mass Spectrophotometry (HPLC-MS/MS). The method detection limit and method quantification limits for triclopyr were reported as 0.005 mg a.i. kg^{-1} and 0.03 mg a.i. kg^{-1}, respectively, with a mean recovery rate of 96 %. The method detection limit and method quantification limits for imazapyr were reported as 0.0006 mg a.i. kg^{-1} and 0.002 mg a.i. kg^{-1}, respectively, with a mean recovery rate of 75 %. A total of 12 duplicate soil samples including four triclopyr and eight imazapyr samples were submitted

for analysis. Relative percent differences ranged from 0-32% and were below the acceptable limit of 50% (<50%) (CCME, 2016).

2.2.5. Statistical Analysis

Sampling intervals at the four sites (CAR, DAW, HJ1, HJ2) installed in 2014 did not allow for dissipation modelling, therefore, herbicide dissipation rates for triclopyr and imazapyr were determined for the LS site (installed in 2015) due to the increased sampling intervals. Additionally, it was expected that the differences in vegetation and soil properties would create differences in soil dissipation rates. Different kinetic models (including first order, 3-parameter biphasic and 4-paramter biphasic) were modeled for each set of herbicide dissipation data. A visual inspection of the data was conducted to examine goodness of fit with the best fitting model with the highest coefficient of determination (R^2) used. The highest R^2 value was assumed to have the best fit for the data.

Triclopyr residues were fit using a 3-parameter biphasic model:

$$C_t = C_0 e^{-kt} + X_0 \qquad \textit{Equation 2.1}$$

where C_t is the herbicide concentration in soil at time t, C_0 is the herbicide concentration at time 0, k is the dissipation rate constant and X_0 is the concentration of the persistent fraction (Langdon et al., 2011).

The three parameter biphasic model was also used to determine the time it took residues to dissipate by 50% ($DT_{50\ BIPHASIC}$) and 90% ($DT_{90\ BIPHASIC}$) of the initial concentration within the first phase, also referred to as the mobile phase (Langdon et al., 2011; Szmigielski et al., 2012). Dissipation values could not be determined for second phase as it identifies the magnitude of the persistent phase (Langdon et al., 2012).

Imazapyr residues from the LS site were fit using a first order kinetic model:

$$C_t = C_0 e^{-kt} \qquad \textit{Equation 2.2}$$

where C_t is the herbicide concentration in soil at time t, C_0 is the herbicide concentration at time 0 and k is the dissipation rate constant (Langdon et al., 2011). After, applying the natural logarithm to both sides of this equation, least squares regression is used to estimate the dissipation constant, k (Wang et al., 2005a). The dissipation rate constant was then used to determine the time

it takes to dissipate by 50% of the initial concentration (DT_{50}) and 90% of the initial concentration (DT_{90}).

Site differences in herbicide residues at each time interval were analyzed using two-way analysis of variance (ANOVA) followed by a TukeyHSD post hoc test. Prior to the ANOVA, data were transformed as needed to meet the assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Bartlett's test). Normality and homogeneity of variance were also confirmed for model residuals. Outliers were checked using the Grubbs test with one outlier identified and removed for the triclopyr data set (9.6 mg kg^{-1} at 3 DAT for the LS dissipation curve). Statistical analyses were completed using the R software (version 3.2.4) (R Core Team, 2016) and dissipation models plotted using SigmaPlot 10.0.

2.3. RESULTS

Comparison of triclopyr and imazapyr residues associated with the different application techniques (cut stump, point injection and backpack spraying) were compared across sites at 30 and 365 DAT (Table 2.3). Of the three application techniques, cut stump application had the lowest residual soil concentrations for both herbicides. Residues for the cut stump treatment were present at 30 DAT (1.3 ± 0.88 mg triclopyr kg^{-1} and 0.05 ± 0.04 mg imazapyr kg^{-1}) but decreased greatly at 365 DAT (0.01 ± 0.01 mg triclopyr kg^{-1} and 0.01 ± 0.002 mg imazapyr kg^{-1}). Triclopyr residues were highest for the point injection treatment at 30 DAT (8.2 ± 8.0 mg a.i. kg^{-1}), however, this was strongly influenced by a single sample at the DAW site (32 mg a.i. kg^{-1}) that likely represented accidental spillage during application. For imazapyr, point injection had the highest concentration of residues (0.54 ± 0.38 mg kg^{-1}) followed by backpack spray (0.12 ± 0.02 mg a.i. kg^{-1}) at 30 DAT. At 365 DAT, all application techniques had similar residual concentrations of imazapyr (0.01 ± 0.002 to 0.03 ± 0.02 mg a.i. kg^{-1}).

Table 2.3. Triclopyr and Imazapyr concentrations (mg a.i. kg d.w.$^{-1}$) for the backpack spray (n=3), cut stump (n=1) and point injection (n=1) treatments at 30 and 365 days after treatment at four sites (CAR, DAW, HJ1, HJ2), as well as, the Mean ± SE for treatment and time interval across all sites. <0.005 indicates concentrations were below detection level for triclopyr. Cut stump and point injection treatments were not analyzed for the LS site. As a result, LS was not included in this table. NA = Not Analyzed.

Herbicide	Site	Treatment					
		Backpack Spray		*Cut Stump*		*Point Injection*	
		30	365†	30	365	30	365
Triclopyr	CAR	3.1	NA	0.13	<0.005	0.34	0.05
	DAW	8.0	NA	3.8	<0.005	32	0.09
	HJ1	2.4	NA	<0.005	0.04	0.04	<0.005
	HJ2	0.42	NA	1.1	<0.005	0.25	0.83
	Mean±SE	3.46 ± 1.61	NA	1.26±0.88	0.01±0.01	8.16±7.95	0.24±0.20
Imazapyr	CAR	0.16	0.01	0.02	0.01	1.6	0.08
	DAW	0.12	0.01	0.002	0.01	0.57	0.02
	HJ1	0.07	0.02	0.16	0.01	0.001	0.004
	HJ2	0.12	0.005	0.03	0.003	0.002	0.01
	Mean±SE	0.12 ± 0.02	0.012 ± 0.003	0.05 ± 0.04	0.01 ± 0.002	0.54 ± 0.38	0.03 ± 0.02

† 365 Days After Treatment samples were not analyzed for triclopyr.

For the backpack spray treatment, the dissipation of triclopyr varied among sites at different time intervals (Figure 2.3). For example, no significant site differences in triclopyr residues were observed 1 DAT but at 30 DAT differences between sites HJ2 and LS were observed (ANOVA, TukeyHSD <0.05). At 30 DAT, slower dissipation was observed at HJ2 with a concentration of 6.60 ± 2.51 mg a.i. kg^{-1} compared to 0.42 ± 0.08 mg a.i. kg^{-1} observed at LS. Correspondingly, HJ2 had high organic matter (17 ± 3.4%) and pH (7.0 ± 0.05), which should lead to higher adsorption of triclopyr. Dissipation of triclopyr applied via backpack spraying was not consistent across the sites with some sites showing a decrease from 1 to 30 DAT (CAR and HJ1), but others showing a slight increase (DAW and HJ2). Additionally, all sites had less dissipation (i.e. higher soil residues) compared with the LS site. Triclopyr residues at 365 DAT were not analyzed as there was little qualitative evidence of triclopyr in vegetation within the treatment plots (Isbister, 2016).

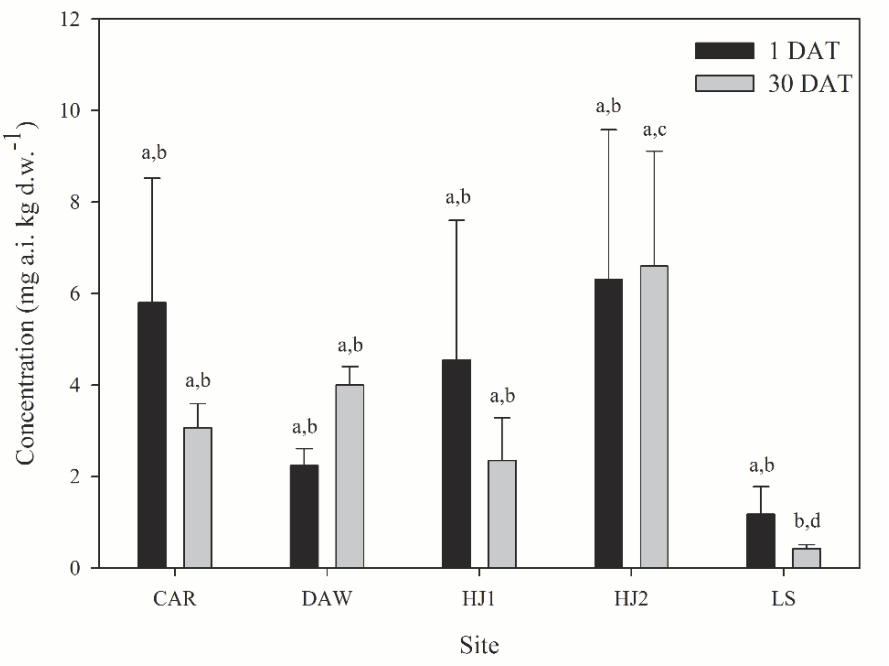

Figure 2.3. Triclopyr residue concentrations from the backpack spray treatment for soils collected from the upper soil horizon (0-3 cm) at 1 & 30 DAT. Bars represent mean ± standard error (n=3) of the estimate and similar letters represent no statistically significant ($p>0.05$) difference between intervals within sites. Only significant difference is between HJ2 and LS at 30 DAT ($p<0.05$).

For imazapyr, a significant decrease in residue concentrations was observed at 365 DAT (0.01 ± 0.002 mg a.i. kg^{-1}) for all sites but there was no significant difference between 1 DAT (0.19 ± 0.05 mg a.i. kg^{-1}) and 30 DAT (0.12 ± 0.02 mg a.i. kg^{-1}). Visual assessment of the data indicated

that HJ1, HJ2 and LS follow similar trends (Figure 2.4). Residue concentrations at CAR and DAW between 1 and 30 DAT increased slightly, but decreased significantly at 365 DAT. No significant site differences were observed for imazapyr residues at 365 DAT applied via backpack spraying.

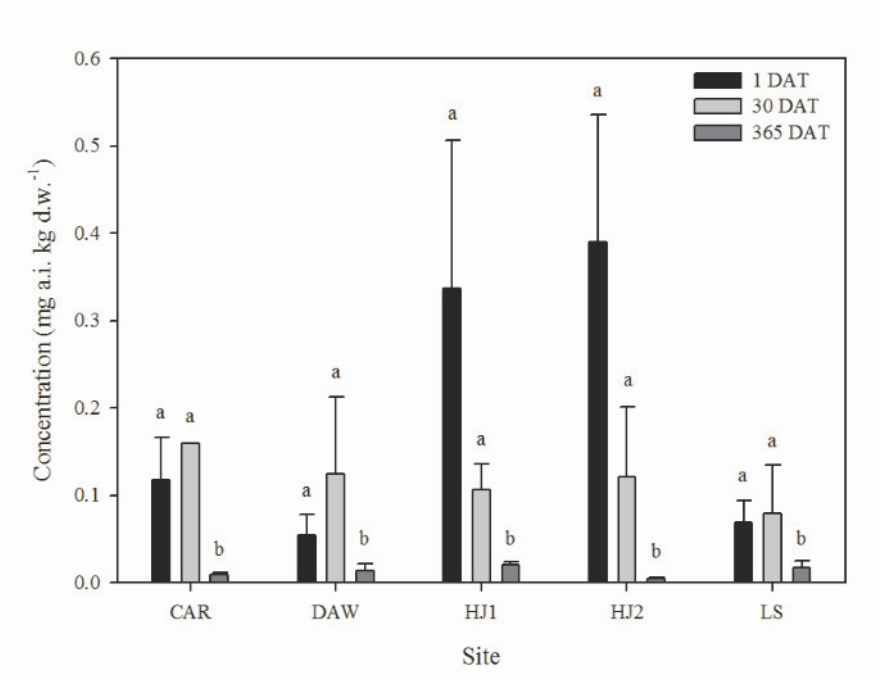

Figure 2.4. Imazapyr residue concentrations (mg a.i. kg d.w.$^{-1}$) for soils collected from the upper soil horizon (0-3 cm) at 1, 30 & 365 DAT from the backpack spray treatment. Bars represent mean ± standard error (n=3) of the estimate and similar letters represent no statistically significant ($p>0.05$) difference between intervals within sites. Significant differences were noted at 365 DAT at each site ($p<0.05$). Among site differences were not observed ($p>0.05$).

Dissipation kinetics of triclopyr and imazapyr were assessed only for the backpack spray application since this application method was expected to have the highest residues in soils representing the worst-case scenario. Triclopyr dissipated much more rapidly than imazapyr in all soils with DT_{50}'s of 1 versus 16 DAT, respectively. Dissipation of triclopyr followed a biphasic model with rapid initial loss observed in the first 3 DAT followed by slow dissipation processes and a persistence of 0.52 mg a.i. kg^{-1} (SE ± 0.18, $p=0.01$) at 60 DAT. (Figure 2.5). In the mobile phase, approximately 50% of the triclopyr had dissipated by one day after treatment ($k=0.76$, SE ± 0.58, $p<0.20$) and 90% had dissipated by three days after treatment at which point the dissipation pattern transitions to the persistent phase. The triclopyr concentration in the persistent phase was 0.52 mg a.i. kg^{-1} indicating a mean loss of 74% of the initial residues (0 DAT). Small sample size

and high variability created uncertainty in the estimates and the dissipation times obtained within the mobile phase should be used with caution.

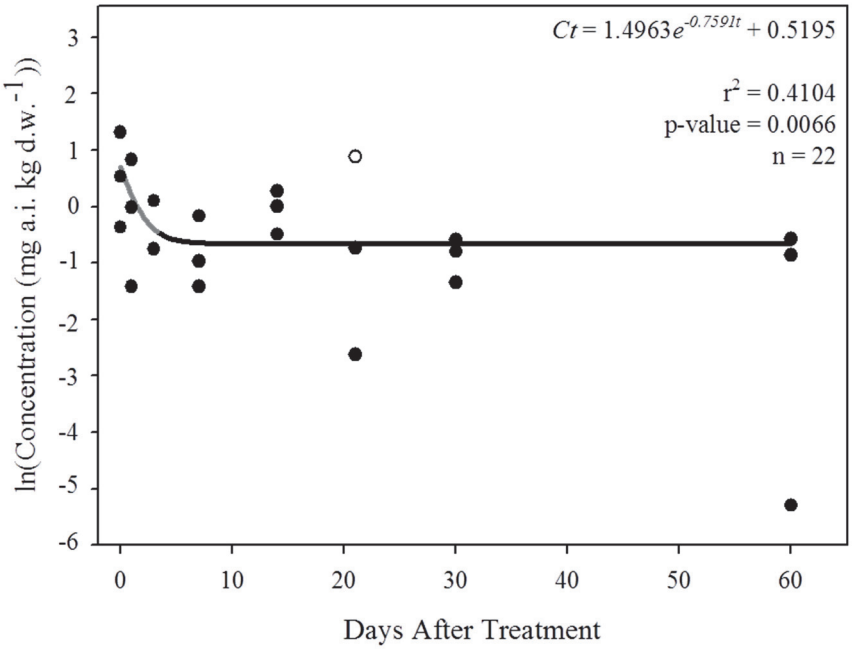

Figure 2.5. Three parameter biphasic dissipation model for triclopyr residues in the upper soil horizon at the LS site (r^2=0.4104). Calculated $DT_{50\ BIPHASIC}$ and $DT_{90\ BIPHASIC}$ values are 1 DAT and 3 DAT, respectively. The concentration in the persistent phase is 0.52 mg a.i. kg^{-1}. Grey line and circles represent the first phase modeled with first order kinetics while the black dots and line represent the persistent phase. The white circle indicates data point that was removed to obtain optimal model fit but was not statistically identified as an outlier.

In contrast to triclopyr, imazapyr dissipation occurred more gradually, roughly following first order dissipation kinetics (Figure 2.6). The degradation constant (k=0.04, SE=0.01, p=0.001) obtained from the linear regression equation was used to determine DT_{50} and DT_{90} values which were 16 and 52 DAT, respectively.

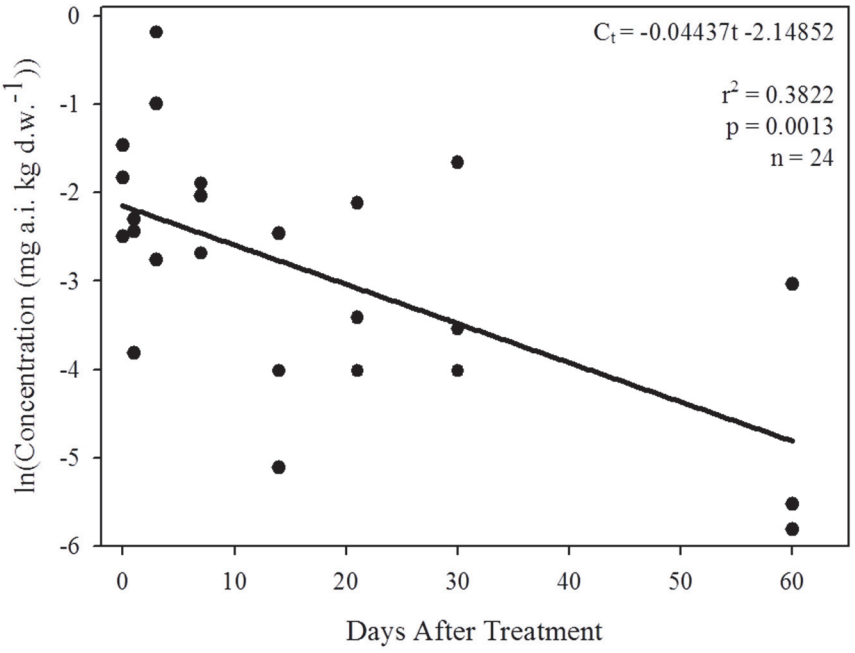

Figure 2.6. First order dissipation model for imazapyr residues from the backpack spray treatment at the LS site (r^2=0.3822) from soils collected from the upper soil horizon (0-3 cm). The DT_{50} and DT_{90} were calculated as 16 and 52 DAT, respectively.

2.4. Discussion

The different treatment applications, cut stump, point injection and backpack spray displayed different dissipation rates. For the cut stump treatment, the concentration of triclopyr was minimal or below the detection limit in most cases. These results support previous studies suggesting the transfer of triclopyr to the soil through root exudation must be minimal (Braverman, 1995; Wahlers et al., 1997). For point injection, the majority of the residues dissipated between 30 and 365 DAT with the exception of the HJ2 soil which increased 3-fold. This could be due to a sampling error or a significant precipitation event that occurred between the intervals that caused herbicide from the foliar surface to transfer to the soil. Imazapyr residues were above the detectable limit for both cut stump and point injection treatments at both time intervals. Residues from both application techniques decreased from 30 to 365 DAT indicating that limited transfer from the vegetation to the soil occurred. Imazapyr residues for the point injection treatment were similar to those observed for the backpack spray application technique and are present at concentrations high enough to effectively control target species and also have a pronounced effect on non-target species (Isbister, 2016). Based on these results, point injection may be a viable alternative to broadcast

application as other studies have indicated that broadcast application affects a larger area than other targeted application techniques (Nowak and Ballard, 2005a). However, there is obviously higher risk associated with the use of imazapyr compared to triclopyr from the perspective of soil persistence and effects to desirable non-target vegetation (Isbister, 2016).

The dissipation rates obtained from the backpack spray treatment at the LS site indicate that triclopyr degraded at a much faster rate than imazapyr along ROWs in the Yukon Territory, which has been similarly documented in temperate regions (Senseman, 2007; Douglass et al., 2016b). Rapid initial dissipation of triclopyr could be a function of surface loss through volatilization and photodegradation which was followed by a persistent phase controlled more by sorption and microbial degradation (Hill and Schaalje, 1985). Imazapyr was more persistent in the soil, perhaps due to lower surface loss. Slower dissipation of imazapyr may have been due to higher sorption and slow microbial degradation processes (Wang et al., 2005a; Gianelli et al., 2014). For both triclopyr and imazapyr, site LS had the lowest residual concentrations when compared to the other sites. Climate data from the Faro weather station near the site indicated 9.6 mm of rain from June 28 to June 30, 2015 so the low concentrations at the site may be due to leaching. Therefore, the dissipation rates listed above should be interpreted with caution. Field trials are highly variable and more work should be done to confirm the effect climatic conditions and soil properties have on the dissipation patterns of these herbicides. This work could include a controlled laboratory study mimicking Yukon Territory climatic conditions, as well as an additional field study with finer sampling intervals including sampling in the winter months.

In Northern Ontario, triclopyr residues had longer residual periods in soil than those in this study with a DT_{50} of 14 days (Stephenson et al., 1990). In Alaska, the majority of triclopyr residues dissipated during the summer months with residues falling below quantification limits within 100 days of herbicide treatment (Newton et al., 2008). In contrast, triclopyr residues in forest soils in Sweden were observed two years after application (Torstenssen and Stark, 1982). Here, triclopyr dissipated much more rapidly than reported in other studies conducted in northern regions but was present at low concentrations for a least one year after application. Faster initial dissipation rates may be attributed to long periods of daylight at the time of application resulting in greater losses via photochemical degradation (Barnes et al., 2009). During the period of application Yukon Territory ROWs were receiving approximately 19 hours of daylight. Some studies have found

comparable dissipation rates to ours with DT_{50} values around 5 DAT for triclopyr (Johnson et al., 1995b; Douglass et al., 2016b). Dissipation in these studies was linked to soil properties including optimal soil moisture, pH > 5, high organic matter and clay content; all of which increase microbial degradation rates (Johnson et al., 1995a; b; Douglass et al., 2016b). Our results showed the only significant site difference in dissipation was between HJ2 and LS at 30 DAT. High residue concentrations were observed at HJ2, a site with higher soil moisture values, soil pH, organic matter and clay contents than LS. These results suggest that soil properties may play a role in the dissipation of triclopyr residues, but further research is needed to understand the degree to which they influence degradation pathways in the Yukon Territory.

Imazapyr is known to have longer residence time in soils than triclopyr with detectable residues observed at 454 and 730 days after application in Alaska and Sweden, respectively (Torstenssen and Stark, 1982; Newton et al., 2008). Imazapyr dissipation in the Yukon Territory followed first order degradation kinetics after backpack application at the LS site with a DT_{50} of 16 DAT which is faster than that typically observed. For example, DT_{50}'s previously reported ranged from 37 to 144 DAT (Börjesson et al., 2004; Wang et al., 2005; Newton et al., 2008; Gianelli et al., 2014; Douglass et al., 2016). In contrast to triclopyr, there were no differences among sites for imazapyr and leaching is thought to be of minimal importance in cold soils (Newton et al., 2008).

2.4.1. Conclusions

Assessment of the backpack spray application indicated intermediate dissipation rates for both triclopyr and imazapyr in the LS soil with DT_{50}'s of 1 and 16 DAT, respectively, but the residue concentrations at LS were consistently lower when compared to the other four sites. While soil properties were not strongly linked to herbicide dissipation in these ROWs, it is possible that this difference could be due to higher sand and lower OM contents, which increased leaching at the LS site. Additional work using a large number of Yukon soils, under controlled climatic conditions typical of a Yukon year, would better delineate the importance of soil properties in the attenuation of herbicides along northern Boreal ROWs. However, understanding the role soil properties play in the attenuation of herbicides is only one piece of the puzzle. Application technique and herbicide selection are also fundamental components of vegetation management programs.

Three techniques (cut stump, point injection and backpack spray) were assessed as part of this research. Backpack spray generally had the highest residues which was to be expected due to its wide application radius. Residues were detected 365 DAT for all three application techniques but at concentrations considerably lower than detected at 30 DAT. Cut stump may be a good technique to employ due to the targeted application of the herbicide and consistently lower residue concentrations in soil than the other techniques assessed. Triclopyr, when compared with imazapyr, appeared to present less potential risk to the environment due to a $DT_{50\ BIPHASIC}$ of 1 DAT and may thus be a better alternative for implementation. However, further studies addressing leaching potential should be completed prior to implementation.

PREFACE: CHAPTER 3

Based on results presented in Chapter 2, triclopyr and imazapyr residues are present in soil at least 365 days after treatment. However, limited information is available surrounding the impact these herbicide residues may have on northern soil ecosystems. Specifically, the toxicity of triclopyr and imazapyr to soil invertebrates found in boreal regions is poorly understood. This data chapter explores the performance of three soil invertebrates (*Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*) and three soil enzymes (phosphatase, arylsulfatase and B-glucosidase) when exposed to triclopyr and imazapyr in organic soils collected at five sites representative of Yukon Territory ROWs. Standardized test protocols were utilized to generate dose-response curves and determine the lethal and effective concentrations that will affect the species tested. The results presented in this chapter will be linked with the dissipation data in Chapter 2 to provide a well-rounded assessment of the risks associated with herbicide application in the Yukon Territory.

3. TOXICITY OF TRICLOPYR AND IMAZAPYR TO INVERTEBRATES IN SOILS REPRESENTATIVE OF YUKON TERRITORY RIGHT-OF-WAYS.

3.1. INTRODUCTION

Soil ecosystems are complex, diverse and heterogeneous systems where soil organisms are an important indicator of ecosystem health (van Straalen, 1998, 2002; Didden and Römbke, 2001; Römbke et al., 2006b; Snyder and Hendrix, 2008). Soil invertebrates are fundamental to the functioning of terrestrial ecosystems by providing several services including the maintenance of soil structure, organic matter decomposition, and nutrient cycling. Changes in abundance and diversity of soil organisms are potential indicators of contaminants influencing ecosystem health (Novais et al., 2010; Römbke, 2014). Ecological risk assessment provides a tool to determine the risks different compounds pose to soil ecosystems (van Straalen, 1998; Didden and Römbke, 2001). Applying ecological risk assessment protocols to natural soils with representative species allows for the calculation of appropriate site-specific use guidelines (Römbke et al., 2006a; b; Princz et al., 2012). Here, we assess the toxicity of two herbicides, triclopyr and imazapyr, used for woody vegetation control along transmission Right-of-Ways (ROWs), to soil organisms in the Yukon Territory, Canada.

Triclopyr is a pyridine equivalent of a phenoxy herbicide that selectively controls broadleaf weeds and woody species (Solomon et al., 1988; Stephenson et al., 1990; Johnson et al., 1995b). It is easily absorbed and translocated through the plant where it mimics the growth hormone auxin, allowing the plant to grow without normal growth regulation processes resulting in phytotoxicity (Pitt et al., 1993; Thompson et al., 2000; Senseman, 2007; Barnes et al., 2009; Grossmann, 2009). Imazapyr is a broad-spectrum herbicide used for control of grasses and broadleaf plants (Wang et al., 2005b; Ramezani et al., 2010; Gianelli et al., 2014). It inhibits acetolactate synthase (ALS) and acetohydroxy acid synthase (AHAS), an enzyme that synthesizes three branched chain amino acids: valine, leucine and isoleucine. At the site of action imazapyr inhibits ALS constraining meristematic growth resulting in plant death (Stidham, 1991; Masson and Webster, 2001; Heiser, 2007). Applications of these herbicides reach the soil surface and remain primarily in upper organic soil horizons (Stephenson et al., 1990; Johnson et al., 1995a; Jourdan et al., 1998; Newton et al., 2008) where they have the potential to influence the soil invertebrate community.

In Canada, the Pest Management Regulatory Agency (PMRA) controls the registration and use of herbicides under the Pest Control Products Act. The registration process requires an environmental risk assessment (ERA) to be performed. As part of the ERA, the environmental fate and toxicity of a herbicide should be evaluated in natural soils, with similar soil types and climatic conditions observed in the proposed use areas (PMRA, 2000). With respect to the toxicological study, earthworms are the only terrestrial invertebrate species required. It is up to the risk assessors to specify other species that should be included based on biodiversity and ecosystem health and sustainability (PMRA, 2000, 2005). Earthworms are not typically found in northern Canada and in many cases are considered highly invasive in the Boreal forest (Addison, 2009; Saltmarsh et al., 2016). Thus, the response of many soil dwelling invertebrates to herbicide exposure in northern soils is largely unknown and the inclusion of ecologically relevant soil invertebrates is necessary (Princz et al., 2012).

Toxicity testing with ecologically relevant species has increased in recent years and includes, among others, *Enchytreaus crypticus* (enchytraeids), *Folsomia candida* (collembola) and *Oppia nitens* (mites) (Römbke et al., 2006b; Princz et al., 2010, 2012). Species in the Enchytraeidae family are abundant in northern Canada (Smith et al., 1990). The test species *E. crypticus* prefers surface organic layers feeding on decomposing plant material and microorganisms (Smith et al., 1990; Didden, 1993; Römbke, 2003). The collembolan, *F. candida,* is an abundant and widespread species (Fountain and Hopkin, 2005). Similar to *E. crypticus*, *F. candida* are decomposers, but prefer to feed on fungal hyphae found within the surface organic layers. Due to their abundance they are also an important prey species for other macro fauna (Fountain and Hopkin, 2005; Environment Canada, 2007b). As decomposers, *O. nitens* are important to northern terrestrial ecosystems by aiding in the development of soil structure (Behan-Pelletier, 1997; Princz et al., 2010, 2012). All of these species are in the same trophic guild (decomposers), but play different roles in the decomposition of organic material (Princz et al., 2012). In addition, these invertebrate species may have an increased role in northern soil ecological communities, when compared to southern communities, because larger macro arthropod species may be absent (Behan-Pelletier, 1997). Incorporating invertebrate species into soil ecological risk assessments will help to ensure soils and the important ecosystem services provided by soils are protected in Canada's North (Römbke et al., 2006b).

Herbicide application also influences soil enzymatic activity. Effects are typically dose dependent and can either stimulate or inhibit enzyme activity (Niemi et al., 2009; Floch et al., 2011). Assessment of herbicidal effect can be broken down into different classes of microorganisms, including sulfur, phosphorus and carbon reducing bacteria, that aid in the cycling of nutrients within the soil ecosystem (Ashman and Puri, 2002). Measurement of soil enzymatic activity via assays can produce highly variable results often linked to the properties of the soil examined (Schäffer, 1993; Gianfreda et al., 1995). The variability produced by enzyme assays in soils with different properties highlights the need to conduct these assays using natural soils.

Natural variation in soil properties influences the dissipation and bioavailability of herbicides regulated primarily though adsorption. Adsorption can be influenced by pH and organic matter content. For example, soils with more acidic pH and higher organic matter contents have stronger sorption capacities slowing dissipation rates and decreasing bioavailability (Johnson et al., 1995a; Jourdan et al., 1998; Szmigielski et al., 2012; Gianelli et al., 2014). Adsorption ultimately defines exposure and response of soil dwelling species to these herbicides (Römbke et al., 2006a; Domene et al., 2011, 2012). Further, the adsorptive properties of soil can possibly lead to an under- or over-estimation of toxicity values (Princz et al., 2010; Domene et al., 2012). Soil dwelling organisms are primarily influenced by soil pH, organic matter content and texture (Kuperman et al., 2006; Römbke et al., 2006a; Domene et al., 2011). The enchytraeid species, *E. crypticus,* is highly sensitive to pH and organic matter content. If the pH is below 4.4 and above 8.2 and organic matter content less than 1% or greater than 28% can have a negative influence on *E. crypticus* performance (Kuperman et al., 2006). Reproduction in *F. candida* can be significantly influenced by soil moisture and texture. Dry soils with higher percentages of fine particles (particularly silt and fine sand) are linked to lower reproduction in *F. candida* (Domene et al., 2011). For *O. nitens,* organic matter is the main soil property affecting reproduction and toxic stress. Increased organic matter leads to increased reproduction of *O. nitens*, likely due to an increase in fungi, a primary food source (Maraun and Scheu, 2000; Princz et al., 2010). Laboratory toxicity tests conducted with site specific soils representative of local soil properties are needed for the development of guidelines protective of terrestrial ecosystem structure and function.

The purpose of this study was to assess the toxicity of triclopyr and imazapyr to soil organisms present in Yukon soils and representative of three boreal ecoregions. The objective was to

determine the concentrations of triclopyr and imazapyr that would adversely impact 25% (EC_{25}) of the soil ecological community in organic soils from five representative sites on Yukon power line ROWs. Specifically, we evaluated the survival and reproduction of three ecologically relevant soil invertebrates, *E. crypticus*, *F. candida*, and *O. nitens*, when exposed to triclopyr and imazapyr. Additionally, the impact of triclopyr and imazapyr on three soil enzymes (phosphatase, arylsulfatase and B-glucosidase) was examined. Obtaining a greater understanding of the toxicity of these herbicides in northern soils using ecologically relevant species and endpoints will aid in the evaluation of the risk associated with herbicide use for transmission ROW vegetation management in the Yukon Territory.

3.2. METHODS

3.2.1. Soil Sampling

Five sites (CAR, DAW, HJ1, HJ2, LS) were selected along transmission ROWs in the Yukon Territory, Canada to assess the toxicity of two herbicides, triclopyr and imazapyr, in northern soils (Table 2.1). Untreated soils were collected from three to five locations at each of the five sites. Each collection site was cleared of vegetation and coarse woody debris prior to sampling. Approximately 20 kilograms (kg) of clean organic soil, typically consisting of the top three centimetres, was collected at each location. Only the organic layer was sampled for the laboratory toxicity tests since the invertebrates selected for the toxicity study prefer this layer (Princz et al. 2012). Furthermore, it is expected that the majority of the herbicide residues will remain in the upper soil horizon (Stephenson et al., 1990; Johnson et al., 2000; Newton et al., 2008; Douglass et al., 2016b). After collection, soils were air dried, sieved to 2 mm, homogenized, and stored at room temperature. Random grab samples (n=5) from the bulk soil for each site were analyzed for soil moisture, total nitrogen, total organic carbon and pH. Soil moisture was analyzed using a Mettler Toledo MJ33 Moisture Analyzer (Mettler Toledo Canada, Mississauga, ON). Total nitrogen was analyzed using the LECO TruMac CNS analyzer (LECO Corporation., St. Joseph, MI), total organic carbon was analyzed using the C-632 LECO Carbon analyzer (LECO Corporation., St. Joseph, MI) and pH was measured using a 0.01M calcium chloride extraction. Table 3.1 summarizes specific soil properties obtained from the organic soil collected at each site. Full soil characterization methodology can be found in Appendix A. Prior to invertebrate toxicity testing

the soil was pasteurized by placing soil in an oven at 80ºC for 48 hours to remove natural fauna from the soils (ie. invertebrate eggs).

Table 3.1. Summary of select properties (mean ± SE) from the top three centimetres, including bulk density, moisture, total nitrogen, total organic carbon and pH, used for laboratory toxicity testing on soil collected from five Right-of-Way sites in the Yukon Territory, Canada.

Site	Bulk Density	Moisture	Total Nitrogen	Total Organic Carbon	pH
	(g/cm^3)	(%)	(%)	(%)	(unitless)
CAR	0.20 ± 0.04	16	1.8 ± 0.02	40 ± 3.3	6.3 ± 0.05
DAW	0.21 ± 0.05	10	1.3 ± 0.01	28 ± 0.75	4.5 ± 0.07
HJ1	0.33 ± 0.08	12	1.2 ± 0.01	22 ± 0.40	6.1 ± 0.07
HJ2	0.44 ± 0.10	6.1	0.81 ± 0.003	13 ± 0.51	7.0 ± 0.10
LS	0.37 ± 0.08	13	0.89 ± 0.012	20 ± 0.93	5.4 ± 0.06

3.2.2. Laboratory Soil Toxicity Tests

Two herbicide formulations commonly used along ROWs 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 isopropylamine salt; BASF Canada Inc., Mississauga, ON). To aid in the dosing of soils field application rates of 4530 g triclopyr ha^{-1} and 720 g imazapyr ha^{-1} were converted to mg a.i. kg $d.w.^{-1}$ using the calculated bulk density for each soil, as listed in Table 3.1 and an assumed sample depth of 3 cm. Each herbicide formulation was used separately with measured concentrations presented in Table 3.2. Nominal concentrations are presented in Appendix B. Soils from the five sites were dosed with a series of eight increasing concentrations of each herbicide (as milligrams of active ingredient per kilogram of soil dry weight [mg a.i. kg $d.w.^{-1}$]) plus a negative control (where no herbicide is added). Each soil was brought to 50 % water holding capacity. Five replicates were included at each dose level. A range finding test using *Folsomia candida* was performed on each soil prior to the definitive test to determine the range where an effect was observed. The range finding tests included five dose levels, including a negative control with three replicates at each level. A surfactant was not used for triclopyr, but Hasten™ Spray Adjuvant (704g L^{-1} Ethyl and Methyl esters of vegetable oil with 196 g L^{-1} non-ionic surfactants; Victorian Chemicals Group, Victoria, AUS) was used for imazapyr. Thus, a surfactant control was included in the imazapyr toxicity tests. Soil toxicity tests (i.e. dose-response tests) were carried out on three soil invertebrates: *Folsomia candida* (collembolan), *Enchytraeus*

crypticus (enchytraeids) and *Oppia nitens* (mites); and with three soil enzymes: phosphatase, arylsulfatase and B-glucosidase.

All invertebrate species were cultured in the Soil Toxicology Laboratory at the University of Saskatchewan, Saskatoon, Saskatchewan. Cultures were kept in the dark at a temperature of 20 ± 2 ºC. Enchytraeids (*E. crypticus)* was cultured in a natural soil with a neutral pH. Rolled oats were added as a food source to the culture as necessary. Cultures of *F. candida* were reared in a plastic culture box with a base of 5:1 plaster of Paris and activated charcoal with baker's yeast added as a food source as needed. Cultures of *O. nitens* were reared in a 125-mL glass jar with a base of 5:1 plaster of Paris and activated charcoal with baker's yeast added as necessary.

Table 3.2. Measured concentrations (mg a.i. kg d.w^{-1}) of triclopyr and imazapyr for each dose level used for the toxicity testing with *Enchytraeus crypticus*, *Folsomia candida* and *Oppia nitens*. Field application rate was calculated using the maximum field application rate (4530 g triclopyr ha^{-1}; 720 g imazapyr ha^{-1}) and the bulk densities (g/cm^3) for each site. Concentrations used were based on the results of the range finding tests. Data points at estimated concentrations were examined to ensure they were within the body of the dose response curve. Nominal concentrations are presented in Appendix B.

Triclopyr

Site	Calculated Field Application Rate (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg d.w.$^{-1}$) for Each Dose Level								
CAR	75.5	0.00	9.01	14.3	36.8	173	347	1399	2728	6725
DAW	73.5	0.00	7.90	10.5	21.0†	31.5	129	387	995	1591
HJ1	45.9	0.00	10.3	17.5	23.0	170	244	1090	1712	2502
HJ2	34.2	0.00	33.6	44.6†	55.6	156	249	1753	2347	4518
LS	41.3	0.00	2.81	4.00	8.09	48.5	75.5	337	514	1114

Imazapyr

Site	Calculated Field Application Rate (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg d.w.$^{-1}$) for Each Dose Level								
CAR	12.0	0.00	1.59	2.70	3.09	4.98	33.1	156	347	6488
DAW	11.7	0.00	1.39	1.82	2.68†	3.54	18.6	105	404	6256
HJ1	7.30	0.00	2.71	4.13	5.28	5.57	242	285	501	10278
HJ2	5.44	0.00	2.71	4.18†	5.65	7.54	36.5	241	722	11532
LS	6.56	0.00	1.48	2.52	4.15	4.99	80.1	106	293	5069

† represents concentrations that were not consistent with intervals and were estimated from adjacent concentrations

Toxicity tests using artificial soil were conducted so comparisons could be made to other published studies where soils with different soil characteristics were used. A standardized laboratory formulation consisting of 10% sphagnum moss (air dried and sieved to 2 mm), 20% kaolin clay, and 70% silica sand was used (Princz et al., 2010; Environment Canada, 2014). Constituents were hand mixed and then allowed to equilibrate for a minimum of three days prior to use in the toxicity tests. Then, if necessary, the pH was adjusted with calcium carbonate to obtain a pH range of 6.0 to 7.5 (Environment Canada, 2014).

Species specific standard operating procedures were followed for all invertebrate species (OECD, 2004; Princz et al., 2010; Environment Canada, 2014). Each test consisted of adding individuals to a glass vessel containing a volume of 30 mL of soil wetted to 50% field water holding capacity. For *F. candida*, 10 to 12-day age synchronized individuals were added to the test vessel, for *E. crypticus* individuals with a well-developed clitellum were used and for *O. nitens* juveniles aged 30 – 42 days were used. Ten individuals were used to start the *E. crypticus* and *F. candida* tests, whereas 15 individuals were selected to start the *O. nitens* tests. This slight deviation from the Princz et al. (2010) protocol was made to increase the number of juveniles produced thus decreasing overall test variability. Test vessels were maintained at approximately 20 ± 2 ºC with 12:12 hour photoperiods for 28 days. Adult survival and reproduction were assessed for each species at 28 days. Due to methodological errors while terminating the *O. nitens* laboratory toxicity assays, results for *O. nitens* are only included for one soil, HJ2.

In addition to invertebrate toxicity tests, assays were conducted to assess the influence triclopyr and imazapyr have on soil enzymatic activity and subsequently on nutrient cycling. Specifically, phosphatase, arylsulfatase and Beta-glucosidase were examined. Phosphatase and arylsulfatase determination followed procedures developed by Eivazi and Tabatabai (1977) and Whalen and Warman (1996), respectively. p-nitrophenol phosphate and p-nitrophenol sulfate were used for the determination of phosphatase and sulfatase, respectively. The B-glucosidase assay was based on procedures developed by Eivazi and Tabatabai (1988) and modified by Arcand (2014). The protocols were modified so only 0.10 g of soil was required for the assay. In addition, procedural controls, with no incubation periods, were run simultaneously to account for any interference that could occur during the assay. Enzymatic activity was assessed using a Bio-Rad iMark Microplate Absorbance Reader (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario) at a wavelength

of 405 nm. Five replicates were included at each dose level. Dose levels for the enzyme assays are presented in Appendix E.

3.2.3. Chemical Analysis

Soil samples were analyzed for triclopyr and imazapyr at the University of Guelph's Food and Agriculture Laboratory using High Performance Liquid Chromatography coupled with tandem Mass Spectrophotometry (HPLC-MS/MS). Soil samples were analyzed to determine measured concentrations at each dose level so that accurate estimates of toxicity could be established. The method detection limit and method quantification limits for triclopyr were 0.005 mg a.i. kg^{-1} and 0.03 mg a.i. kg^{-1}, respectively, with a mean recovery rate of 96 %. The method detection limit and method quantification limits for imazapyr were 0.0006 mg a.i. kg^{-1} and 0.002 mg a.i. kg^{-1}, respectively, with a mean recovery rate of 75 %. Twelve doses levels were analyzed in triplicate to ensure consistency in the dosing procedure. Full analytical results are presented in Appendix B.

3.2.4. Statistical Analysis

Survival and reproduction endpoints were generated from the invertebrate toxicity tests. Survival was normalized as a ratio of the mean of the control, whereas the total number of juveniles produced represented the reproduction endpoint. In replicates where survival was greater than the mean of the control, values were adjusted to equal the mean of the control (ie. corrected to a value of 1). This was done to aid in model fitting for the survival endpoint. Outliers were assessed visually with six outliers identified that were subsequently verified with a Grubb's outlier test. One additional point, from the CAR triclopyr *F. candida* test, was removed due to a convergence failure. The points were removed from the data set for a total of 958 data points.

Dose response curves were generated using a three-parameter Weibull function apart from two triclopyr endpoints. Enchytraeid, *E. crypticus,* reproduction in the LS soil and *F. candida* survival in the HJ2 soil, which were modelled with a three-parameter log-logistic and three-parameter log-normal functions, respectively. The model coefficients and associated p-values were assessed for each model. Lack of fit was assessed using the 'modelFit' function in the 'drc' package in R, which compares the dose-response model to a general one-way ANOVA using an F-test. (p-value>0.05 indicates acceptable fit). Models with an poor fit ($p<0.05$) were visually assessed to determine if fit (within the 95% confidence intervals) was adequate. The 28-day lethal concentrations for 10%

or 25% (28-d LC_{10}) and 25% (28-d LC_{25}) for survival and the effective concentration for 10% (28-d EC_{10}) and 25% (28-d EC_{25}) for reproduction, along with the 95% confidence intervals, were determined using the 'ED' function in the 'drc' package for dose response modeling. Dose-response modeling was conducted using the 'drc' package in R software (version 3.2.4) (Ritz et al., 2005, 2015; R Core Team, 2016).

Dose-response could not be modelled for the enzyme assays (i.e. phosphatase, arylsulfatase and B-glucosidase) due to a lack of toxic response. Site differences were examined for each of the enzymes assessed using analysis of variance (ANOVA) followed by a TukeyHSD post hoc test using the negative control (no herbicide) data only. Prior to the ANOVA, data was transformed to meet the assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Fligner-Killeen test). Normality and homogeneity of variance were also confirmed for model residuals.

For *E. crypticus* and *F. candida*, a nested ANOVA (with site soils as a covariate) was used to compare the means for the negative (no herbicide) and Hasten (surfactant) controls conducted for the imazapyr laboratory toxicity tests. QQ plots and fitted vs. residual plots were checked to verify normality and equal variance. Least squares means ('lsmeans') was used post hoc to confirm significant differences ($p<0.05$) between Hasten and the control at each site. For *O. nitens* at HJ2 a t-test was used to compare the means for the negative (no herbicide) and Hasten (surfactant) controls.

All statistical analyses were completed in R statistical software (version 3.2.4) (R Core Team, 2016). Figures were generated using both R (Figure 4.2, Figure 4.3 and Figure 4.4) and SigmaPlot 10.0 (Figure 4.1 and Figure 4.5).

3.3. RESULTS

The impact of triclopyr and imazapyr on ecologically relevant soil organisms in typical soils found along ROWs in the Yukon Territory was examined. All 28-d LC_{25} and 28-d EC_{25} values were above concentrations that could be expected from the manufacturer recommended maximum application rates. For triclopyr, the lowest 28-d LC_{25} was observed for *E. crypticus* in the DAW soil and the lowest EC_{25} was observed for *F. candida* reproduction in the LS soil (Figure 3.1). The lowest 28-d LC_{25} and 28-d EC_{25} values for imazapyr were found for *E. crypticus* but in the CAR and DAW soils respectively. More sensitive endpoints, 28-d LC_{10} and 28-d EC_{10}, were also utilized

to draw conclusions about the toxicity of these herbicides along northern ROWs. All invertebrate toxicity tests met validity criteria (greater than 80% adult survival, greater than 10 juveniles produced) as stated in the standardized protocols (OECD, 2004; Princz et al., 2010; Environment Canada, 2014). Model parameters and effective concentrations are presented in Appendix C with dose response figures for all data presented in Appendix D. Data sets where no model was adequately fit are also presented in Appendix D to show the distribution of data points.

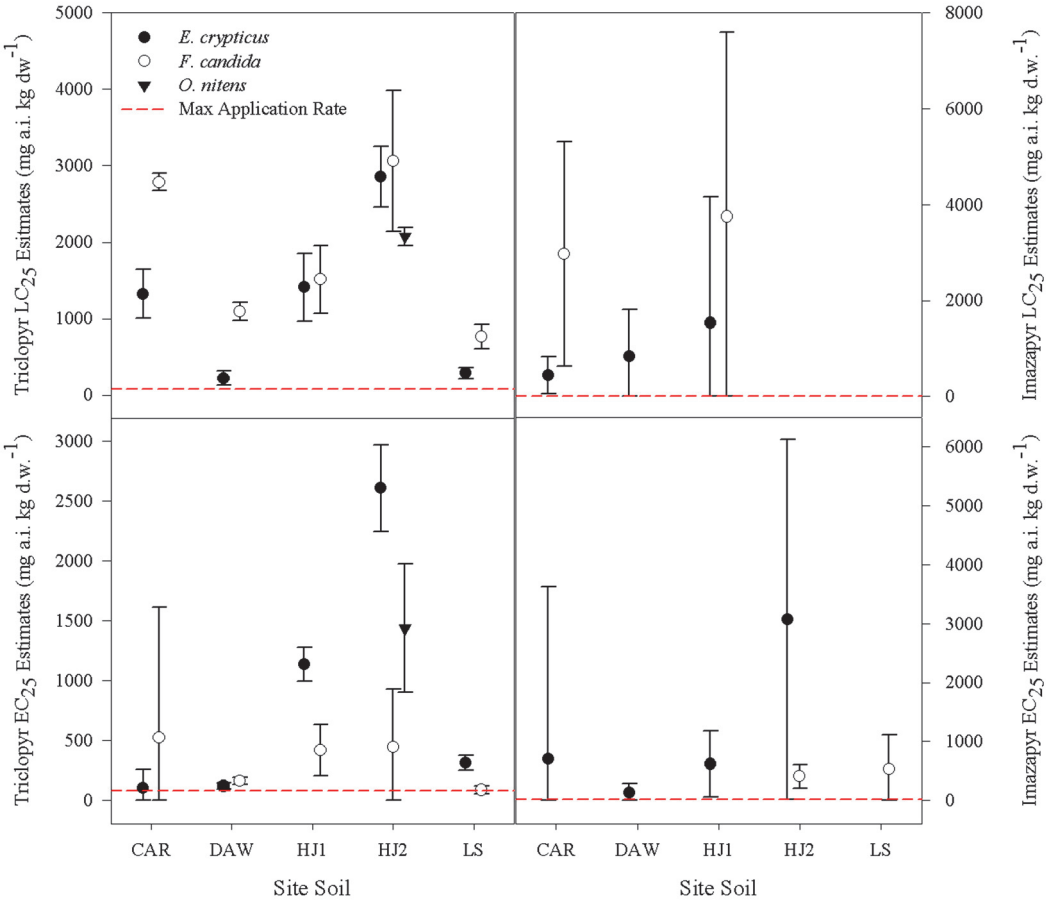

Figure 3.1. Summary of the effect of triclopyr and imazapyr on adult survival (28-d LC_{25}) and the number of juveniles produced (28-d EC_{25}) for *Enchytreaus crypticus*, *Folsomia candida*, and *Oppia nitens* in organic soils collected at five ROW sites in the Yukon Territory, Canada. Triclopyr results are on the left and imazapyr are on the right. The 28-day lethal concentration for 25% (28-d LC_{25}) survival values are in the top panels with 28-day effective concentration for 25% (28-d EC_{25}) reproduction values in the bottom panels. Symbols represent the species with bars representing the 95% confidence intervals. Negative lower confidence intervals were corrected to 0. The red dashed line represents concentrations expected from the max application rates of 75.5 mg triclopyr kg d.w.$^{-1}$ and 12.0 mg imazapyr kg d.w.$^{-1}$. Toxicity endpoints are missing where a model could not be fit and an 28-d LC_{25}/28-d EC_{25} could not be estimated. Imazapyr 28-d LC_{25} for *Oppia nitens* in the HJ2 soil was omitted from the figure due to high values well above the maximum application rate and other 28-d EC_{25} values.

Triclopyr had minimal impacts on the toxicity estimates for the survival of *E. crypticus*, *F. candida* and *O. nitens* (HJ2 only) with all 28-d LC_{25} and 28-d EC_{25} values above the maximum field application rate (Figure 3.1). Reproduction was more sensitive than survival with 28-d EC_{10} values for *E. crypticus* (18 ± 21.3 mg triclopyr kg d.w.$^{-1}$ in the CAR soil) and *F. candida* (34 ± 9.97 mg triclopyr kg d.w.$^{-1}$ in the LS soil) below the concentrations expected from the calculated maximum field application of 75.5 mg triclopyr kg d.w.$^{-1}$ (Appendix C). The 28-d EC_{10} value for *E. crypticus* (76.0 ± 17.8 mg triclopyr kg d.w.$^{-1}$) in the DAW soil was just above the maximum field application rate. Triclopyr had minimal impact on the survival and reproduction of *O. nitens* in the HJ2 soil with 28-d LC_{10} (1874 ± 98.4 mg triclopyr kg d.w.$^{-1}$) and 28-d EC_{10} (1115 ± 353 mg triclopyr kg d.w.$^{-1}$) values well above the maximum field application rate (Figure 3.2).

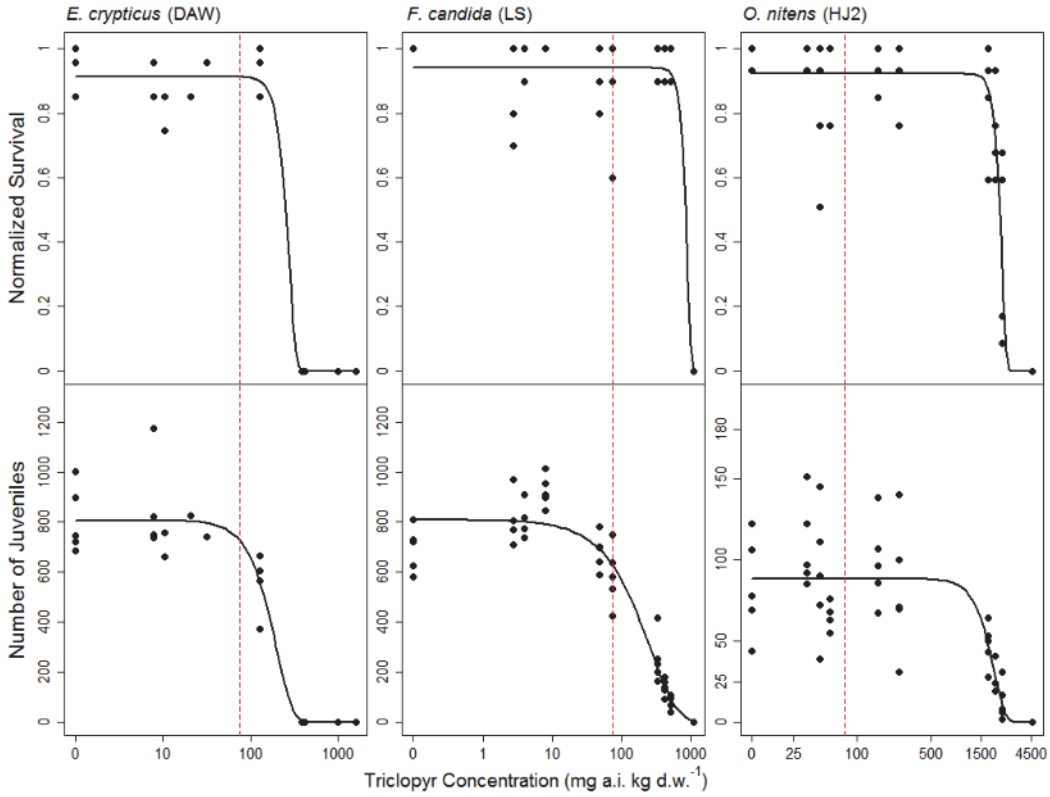

Figure 3.2. Response of *Enchytreaus crypticus, Folsomia candida* and *Oppia nitens* survival (normalized by mean of control) and reproduction (number of juveniles) to triclopyr in select Yukon Territory soils. Dots represent individual data points while the line represents the three parameter Weibull distributions in DAW, LS and HJ2 soils. The red dashed line represents the concentrations expected from maximum application rate of 75.5 mg triclopyr kg d.w.$^{-1}$.

Enchytraeid, *E. crypticus,* survival and reproduction in triclopyr dosed artificial soil was highly variable and no dose-response relationship could be adequately modelled. Survival of *F. candida*

in artificial soil (28-d LC_{10} =393 ± 72.0 mg triclopyr kg d.w.$^{-1}$) was above the concentrations expected from the mean application rate and was comparable to the 28-d LC_{10} values calculated for the site soils. The *F. candida* reproduction endpoint, however, was below the concentrations expected from the maximum field application rate with a 28-d EC_{10} value of 8.65 ± 3.30 mg triclopyr kg d.w.$^{-1}$.

Imazapyr concentrations had some effect on survival for all invertebrate species tested but 28-d LC_{25} values above the concentrations expected from the maximum field application of 12 mg imazapyr kg d.w.$^{-1}$ (Figure 3.1). Even at the 28-d EC_{10} level, no toxicity endpoints were below the field application rate. The most sensitive response was observed for *E. crypticus* in the DAW soil with an 28-d EC_{10} of 23.3 ± 21.3 mg imazapyr kg d.w.$^{-1}$, a value nearly double concentrations expected from the maximum field application rate (Figure 3.3). The reproduction data for *O. nitens* was highly variable and could not be adequately modelled for dose-response. However, a visual assessment of the data indicates little impact on reproduction at relevant field concentrations (Figure 3.3). For artificial soil, only *F. candida* was tested with imazapyr. Reproduction and survival for *F. candida* were above the concentrations expected from the maximum field application rate with 28-d EC_{10} and 28-d LC_{10} values of 315 ± 71.3 mg imazapyr kg d.w.$^{-1}$ and 266 ± 209 mg imazapyr kg d.w.$^{-1}$, respectively.

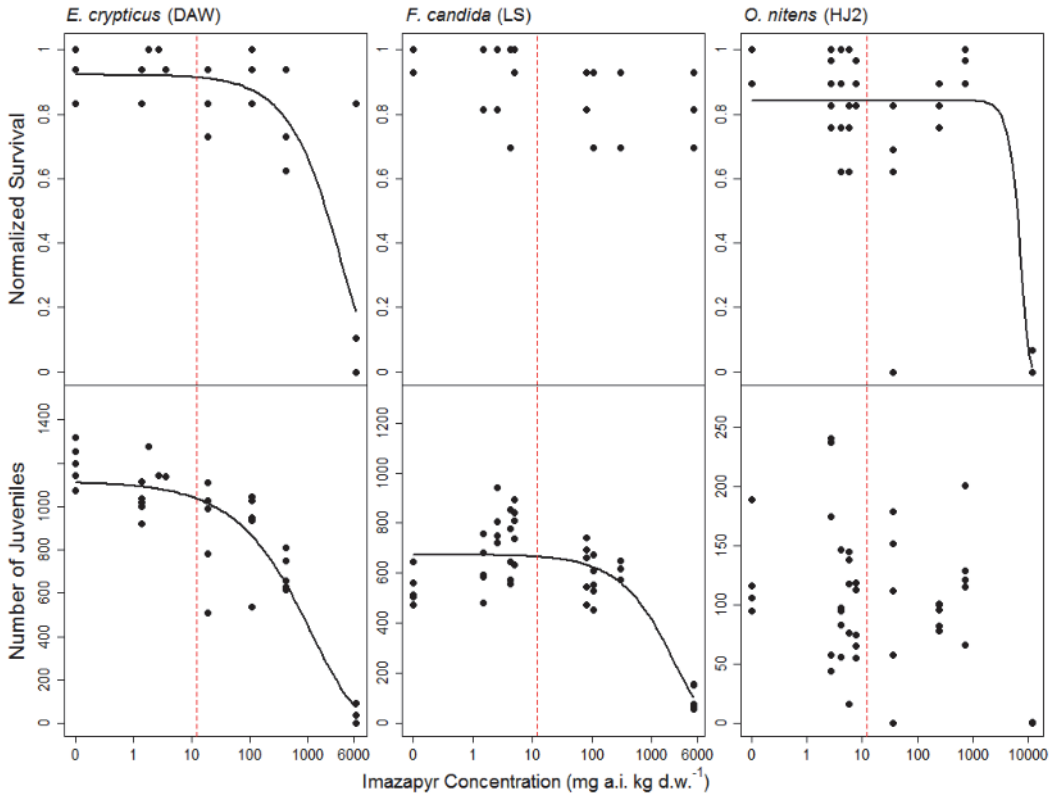

Figure 3.3. Response of *Enchytreaus crypticus, Folsomia candida* and *Oppia nitens* survival (normalized by mean of control) and reproduction (number of juveniles) to imazapyr in select Yukon Territory soils. Dots represent individual data points while the line represents the three parameter Weibull distributions in DAW, LS and HJ2 soils. The red dashed line represents the concentrations expected from maximum application rate of 12.0 mg imazapyr kg d.w.$^{-1}$. *F. candida* survival in the LS soil and *O. nitens* reproduction in the HJ2 soil, responses were highly variable and could not be modelled.

Preliminary enzyme assays indicate that triclopyr and imazapyr residues have little impact on phosphatase, sulfatase and B-glucosidase activity. Soil enzymatic activity could not be adequately modelled for dose response (Appendix E). However, site specific differences in activity, based on the negative control data (i.e. no herbicide), were observed for each of the three enzymes (phosphatase, arylsulfatase and B-glucosidase) tested (Figure 3.4). Phosphatase activity was significantly higher in the HJ2 soil compared to the other three sites tested (ANOVA, TukeyHSD<0.05). HJ1 and HJ2 also had the highest arylsulfatase activity while CAR soil had significantly lower activity (ANOVA, TukeyHSD<0.05). The HJ1 soil had the highest B-glucosidase activity while HJ2 had the lowest B-glucosidase activity.

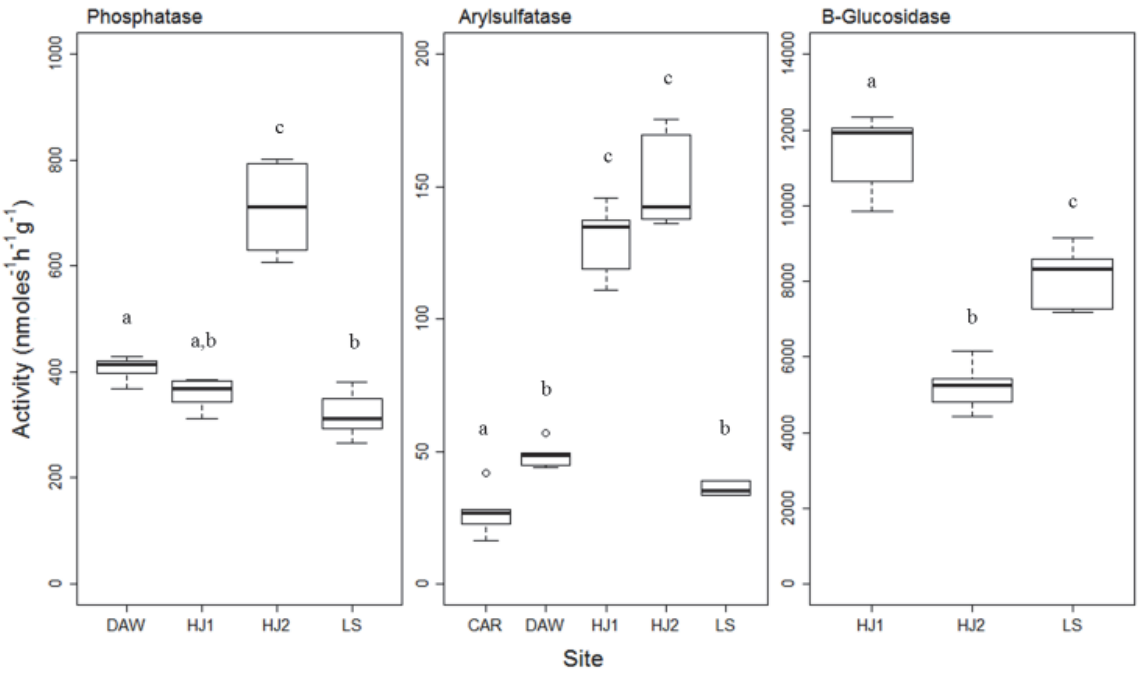

Figure 3.4. Site-specific differences in soil phosphatase, arylsulfatase and B-glucosidase activity measured as nmoles $h^{-1}g^{-1}$ relative to the negative control data (i.e. no herbicide). Thick horizontal lines represent the median value while the box represents the lower and upper quartiles (25 and 75%). The whiskers represent the maximum or minimum data points while dots represent possible outliers. Different letters represent significant differences in enzymatic activity between sites (ANOVA, TukeyHSD>0.05).

A surfactant control was included in the 28-day toxicity assays to ensure that the Hasten (surfactant) solution was not influencing the toxicity of imazapyr to *E. crypticus*, *F. candida* and *O. nitens* when compared to the negative (no herbicide) control. Hasten had no effect on the survival or reproduction of *O. nitens,* but significant effects ($p<0.05$) were observed for *E. crypticus* and *F. candida* (Figure 3.5). The greatest reduction in survival and reproduction due to Hasten was observed in the DAW soil. Survival was also lower in the Hasten controls for *E. crypticus* and *F. candida* in the HJ1 and HJ2 soils, respectively. Enchytraeid (*E. crypticus)* reproduction in the LS soil and *F. candida* reproduction in the LS soil were the only tests to show significant increases ($p<0.05$) in the Hasten control when compared to the negative control. Artificial soil was only tested for *F. candida* where Hasten had no effect on survival, but juvenile production was significantly lower ($p<0.05$) than observed in the negative control.

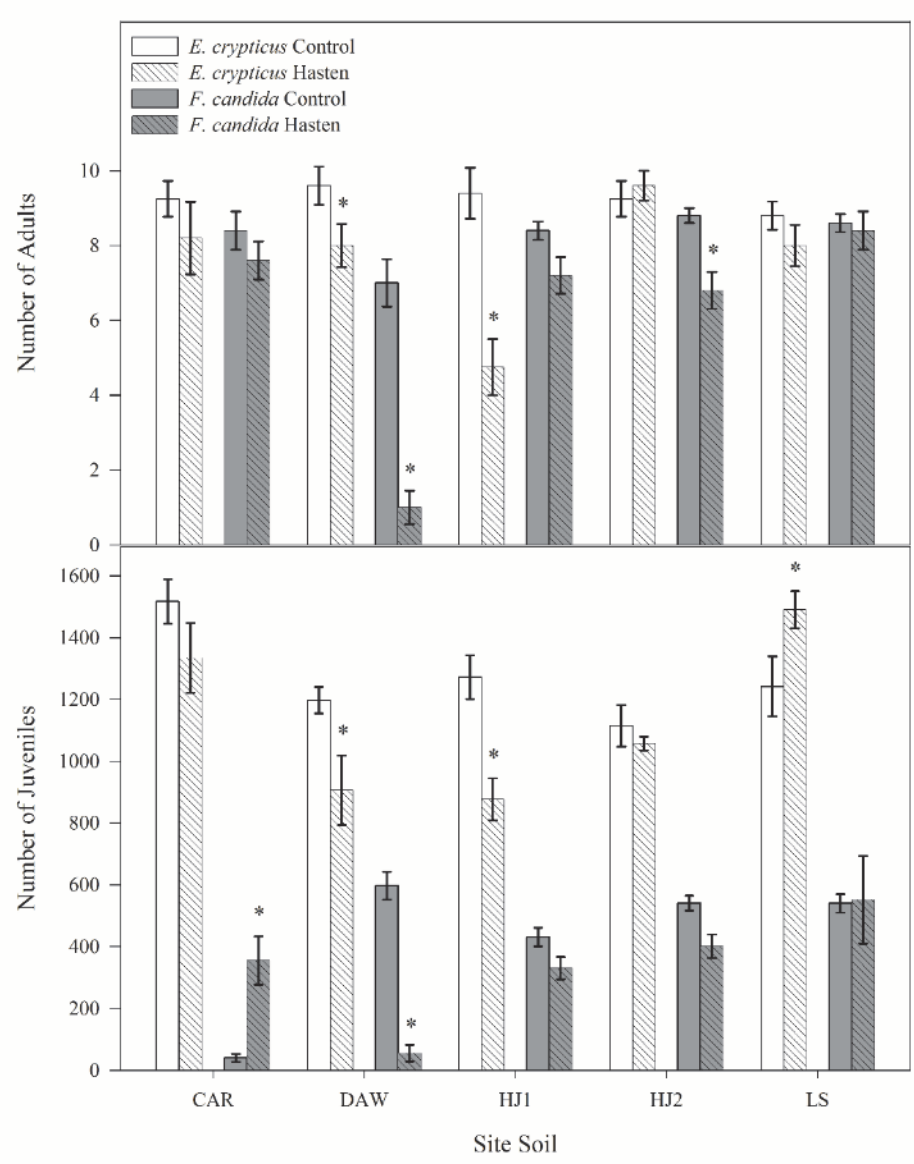

Figure 3.5. Mean adult survival and total juveniles produced in the negative (no herbicide) and Hasten (surfactant) controls from the *Enchytreaus crypticus* and *Folsomia candida* 28-day toxicity assays from five site soils. Error bars represent the standard error of the mean (n=5). (*) represents a significant difference (least squares means, $p<0.05$) between the Hasten control and the associated negative control.

3.4. DISCUSSION

The response in soil enzymatic activity and soil dwelling invertebrates to herbicide exposure in Yukon Territory soils was minimal. Even at sensitive thresholds, such as 28-d LC_{10} and 28-d EC_{10}, effects were generally not observed below concentrations expected from the maximum field application rate. Further, results from the dissipation study (Chapter 2) indicated that the soil environmental concentrations at 1, 30 and 365 days after treatment are lower than the calculated

maximum field application concentrations likely due to the herbicides being intercepted by vegetation prior to reaching the soil surface. Based on the known mechanisms of action for both triclopyr and imazapyr, minimal toxic responses were expected for the soil invertebrates exposed to the herbicides. Triclopyr mimics the growth hormone, auxin, while imazapyr inhibits the enzymes acetolactate synthase (ALS) and acetohydroxy acid synthase (AHAS); auxin and ALS are only found in plants (Pitt et al., 1993; Senseman, 2007; Barnes et al., 2009; Grossmann, 2009; Stidham, 1991; Masson and Webster, 2001; Heiser, 2007). For both triclopyr and imazapyr, no information is available for the toxic action in soil invertebrates. Both herbicides are organic compounds and, similar to other organic compounds, it is likely that the soil organisms tested can biotransform the herbicides into less toxic by-products up to a certain concentration (Stroomberg et al., 2003, 2004; Paumen et al., 2008). The results summarized above indicate that the threshold levels at which toxic responses are observed are above concentrations expected from the maximum field application rate but different thresholds exist for different species.

While direct toxicity of these herbicides to the soil organisms tested is minimal, terrestrial organisms can be indirectly affected by herbicide application. For instance, reduced vegetative cover can alter predator prey dynamics in terrestrial systems (Freemark and Boutin, 1995). Soil dwelling organisms are more influenced by habitat quality, reflected in the soil properties, which can subsequently affect their sensitivity to environmental contaminants. This ultimately increases the complexity associated with estimating the risk of chemicals in soil ecosystems (Didden and Römbke, 2001; Højer et al., 2001). Therefore, a greater understanding of the role of field conditions, such as soil properties and climatic conditions, play in the sensitivity of organisms to environmental contaminants is critical when assessing soil ecological risk.

3.4.1. Influence of Soil Properties

Understanding the influence soil properties have on the toxicity of chemicals to organisms is a fundamental component of soil ecotoxicology (van Straalen and Denneman, 1989; van Straalen, 2002). Adsorption is one of the most important processes regulating the bioavailability and thus the toxicity of organic compounds to soil organisms (van Straalen and Denneman, 1989; van Straalen, 2002). Two of the largest drivers of adsorption are pH and soil organic matter (van Straalen and Denneman, 1989; Crommentuijn et al., 1997; Ponge et al., 2002; van Straalen, 2002; Amorim et al., 2005b, 2008; Princz et al., 2012). Soft bodied invertebrates, such as *E. crypticus*,

are known to be highly sensitive to changes in pH with an optimal range of 5.1 to 7.4 (Amorim et al., 2005b; Kuperman et al., 2006). Slightly acidic soil pH was observed below the optimal range in the DAW (4.5 ± 0.07) and LS soils (5.4 ± 0.06). The lowest 28-d EC_{10} values for triclopyr (*E. crypticus* and *F. candida* reproduction) were observed in the DAW (76 ± 17.8 mg triclopyr kg $d.w^{-1}$) and LS (34 ± 9.97 mg triclopyr kg $d.w^{-1}$) soils. The lowest 28-d EC_{10} values for imazapyr (*E. crypticus* and *F. candida* reproduction) were also observed in the DAW (23.3 ± 21.3 mg imazapyr. kg $d.w.^{-1}$) and LS soils (156 ± 129 mg imazapyr. kg $d.w.^{-1}$),

Organic matter is another soil property that influences the response of soil organisms exposed to triclopyr and imazapyr. All of the soil invertebrate species (*E. crypticus, F. candida* and *O. nitens*) tested here reside primarily in the upper soil horizon with soil organic content being important to their reproductive success (Jänsch et al., 2005; Princz et al., 2012). Organic matter, as measured by total organic carbon (TOC) content, was highest in the CAR (40 ± 0.33%) and DAW (28 ± 0.75%) soils when compared to the other three sites. For imazapyr, *E. crypticus* reproduction was the most sensitive endpoint in the DAW soil. The highest 28-d EC_{10} values for both herbicides were generally observed in the HJ2 soil, the soil with the lowest TOC content (13 ± 0.51%).

Both triclopyr and imazapyr are organic acids, which are known to only weakly sorb to soil colloids. Weak sorption occurs since the anions of the herbicide's functional groups are not attracted to anionic colloidal surfaces (Ashman and Puri, 2002). However, low soil pH increases sorption of these herbicides, via decreased ionization, ultimately reducing bioavailability. Lower pH soils in this study tended to have the lowest 28-d EC_{10} toxicity estimates, therefore reduced bioavailability may not be occurring in these soils. The lower soil pH may have resulted in less optimal habitat influencing reproduction capacity of the invertebrates. These results are consistent with Kuperman et al. (2006) who found organic matter contents greater than 28% had a negative impact on *E. crypticus* reproduction. The reason for the decreased reproduction is unknown since higher organic matter values should result in reduced bioavailability (Kuperman et al., 2006; Domene et al., 2012). Domene et al. (2012) found that organic matter played a limited role in the reproduction of *F. candida*. While it is possible that the acidic pH and high organic matter contents could enhance the toxicity of triclopyr and imazapyr to the soil organisms tested, it is more likely that these parameters resulted in sub-optimal habitats that indirectly influenced the toxicity results.

The different responses of soil invertebrates to herbicides across a range of soil properties indicates that further research is needed to determine which soil properties play a role in the toxicity of triclopyr and imazapyr to soil dwelling organisms (Amorim et al., 2005a; Kuperman et al., 2006; Domene et al., 2012).

3.4.2. Soil Enzymatic Activity

Soil enzymatic activity, and ultimately nutrient cycling, is highly variable when exposed to herbicides with inhibition, stimulation or limited effects possible. Highly variable enzyme activity is often linked to both the herbicide's chemical properties and the properties of the soils examined (Schäffer, 1993; Gianfreda et al., 1995; Sannino and Gianfreda, 2001; Floch et al., 2011; Riah et al., 2014). Herbicides can influence enzyme activity both directly, by interacting with protein groups on the enzymes, and indirectly, by serving as a potential source of carbon, phosphorous and nitrogen for soil microorganisms (Tabatabai, 1994; Floch et al., 2011). By examining soil enzymatic activity, it may be possible to determine which groups of microorganisms are responsible for herbicide degradation, as well as, how herbicides influence nutrient cycling.

Phosphatase, arylsulfatase and B-glucosidase assays showed limited dose-response relationships indicating that overall nutrient cycling is likely not impacted by exposure to triclopyr and imazapyr at these concentrations. However, when comparing the negative (no herbicide) controls, clear site differences were evident. Phosphatase activity was highest in the HJ2 soil, a soil with a comparatively neutral pH (7.0 ± 0.10). The LS and DAW soils, with acidic soil pH, had the lowest phosphatase activity indicating that pH may be driving phosphatase activity in the soils examined. For arylsulfatase and B-glucosidase no clear trends were evident. A relationship between enzyme activity and pesticide contamination appears to exist but further studies are needed to fully understand the drivers of that relationship.

3.4.3. Role of Formulations and Surfactants

Formulations and surfactants may contribute to the toxicity of herbicides to soil organisms. Surfactants or adjuvants are often added to spray mixtures to enhance the dispersal properties of herbicides, ultimately enhancing adsorption at the plant surface (Hazen, 2000; Krogh et al., 2003; Liu, 2004). Pereira et al. (2009) compared the toxicity of active ingredients to their commercial formulations and found that in some cases the toxicity of the active ingredient under- or over-

estimated the toxicity of commercial formulations highlighting the need to test formulations to understand relevant responses. For aquatic organisms, a comparison of the herbicide propanil and its commercial formulation Stam Novel Flo 480 indicated that the toxicity associated with the formulation was underestimated for *Daphnia magna* (freshwater cladoceran), but overestimated for the *Pseudokirchneriella subcapitata* (freshwater green algae) (Pereira et al., 2009). Further, non-target aquatic organisms and vegetation can have different toxic responses when exposed to different glyphosate formulations likely due to the 'inert' ingredients in the formulations (Sihtmäe et al., 2013). These results highlight the importance of understanding the effects of both the active ingredient and the associated formulations or surfactants to obtain realistic estimates of risk associated with herbicide use.

Hasten, the surfactant used with imazapyr, is formulated with an esterified blend of canola and corn oil, as well as, unspecified non-ionic surfactants (Victorian Chemical, 2015; Kleinhenz et al., 2016). To identify if Hasten was influencing the toxicity of imazapyr, a surfactant control (Hasten only) was added to the toxicity testing for imazapyr. Overall, there were contradictory results among the different soils. For example, significant decreases in *E. crypticus* and *F. candida* survival and/or reproduction were observed in the surfactant controls when compared to the negative controls in the DAW and HJ1 soils and the DAW and HJ2 soils, respectively. However, significant increases in reproduction were observed in the LS and CAR soils. The observed decreases in the DAW soil could be due to the surfactant alone or the surfactant could be acting in conjunction with the low soil pH to increase the sensitivity of *E. crypticus* and *F. candida*. A study with saltwater marsh invertebrates indicated that Hasten was less toxic to two aquatic annelid species (*Lumbriculus variegatus* and *Aglaophamus australiensis*) than Fusilade Forte formulation (active ingredient: fluazifop-p-butyl) (Kleinhenz et al., 2016). In contrast, these results indicate that *E. crypticus* and *F. candida* have a higher sensitivity to Hasten than to the Hasten/Arsenal Powerline (imazapyr) mixture. Hasten has not been studied extensively but other non-ionic surfactants have been shown to cause DNA damage, oxidative stress and narcosis in *Escherichia coli* (Nobels et al., 2011). However, little toxicity was present at the concentration at which 20% of the test species were affected (EC_{20}) indicating that a threshold concentration must be obtained before toxic stress occurs (Nobels et al., 2011). While testing needs to be conducted on invertebrate species, oxidative damage as a toxic mechanism could influence subsequent generations (Paumen

et al., 2008). Multigenerational studies should also be conducted to determine the long-term influence of active ingredients, formulations, and surfactants to soil organisms.

3.5. CONCLUSIONS

The environmental fate and toxicity of herbicides must be evaluated prior to registration, as per the Canadian Pest Control Products Act. With respect to the toxicological evaluation, earthworms are the most commonly assessed soil-dwelling organism, with other species and groups under-represented. Therefore, knowledge of the toxicity of chemicals to a range of soil organisms in natural soils is limited. Here, we assessed the toxicity of two herbicides (triclopyr and imazapyr) to three soil invertebrates (*E. crypticus*, *F. candida* and *O. nitens*) and three soil enzymes (phosphatase, arylsulfatase and B-glucosidase). When only taking the organisms tested here into account, the risk associated with triclopyr and imazapyr use for vegetation management in the Yukon Territory appears to be minimal. Results from the phosphatase, arylsulfatase and B-glucosidase assays showed limited dose response relationships indicating that overall nutrient cycling is likely not impacted by exposure to triclopyr and imazapyr at normal application rates. However, clear site differences, based on soil properties, were evident.

Prior to herbicide application the soil properties at each site should be examined to determine if greater toxic responses are expected. Further work should be conducted with these invertebrate species to determine which soil properties may be influencing the toxicity of each herbicide. Additionally, multi-generational studies to evaluate the long-term risks of these herbicides on soil invertebrates would be beneficial. Further studies should also be conducted to fully understand the relationship between soil enzymatic activity and triclopyr and imazapyr exposure. Understanding the modifiers of toxicity could aid in the development of site-specific application guidelines aimed at reducing the risk of vegetation control measures along ROWs in the Yukon Territory.

4. SYNTHESIS: LINKING HERBICIDE DISSIPATION TO SOIL ECOLOGICAL RISK

4.1. INTRODUCTION

Integrated vegetation management (IVM) is an adaptive approach that utilizes information from different subject areas such as herbicide fate and toxicity, soil characteristics, structure and function of vegetative communities, and public perceptions to develop comprehensive vegetation control programs (Nowak and Ballard, 2005b). IVM practices often combine different techniques including mechanical, chemical, and biological methods, to effectively control target species while also minimizing impacts on the environment (Nowak and Ballard, 2005a; Yahner, 2006; Thiffault and Roy, 2011; Douglass et al., 2016b). Mechanical techniques, including brushing and mowing, were used to control target species until the 1940s (McLoughlin, 2014). During the chemical revolution of the 1950s, the broadcast application of herbicides became the norm in North America due to their effectiveness against target species (McLoughlin, 2014). In recent years, selective management, the targeting of problem areas and species, has grown in popularity amongst vegetation managers. Selective management decreases the chemical load on the environment and can include both selective removal and native plant seeding (Nowak and Ballard, 2005b; McLoughlin, 2014). The use of IVM practices in northern latitudes has been minimal due to the lack of understanding of the long-term effectiveness on northern target species and a lack of documented trials of IVM with boreal species.

In the North, vegetation management currently consists of removing tall, fast growing woody vegetation such as *Populus spp*. and *Salix spp*. via brushing and mowing techniques. While mechanical control techniques do not increase chemical load in the ecosystem they are not practical long-term. When cut, target species grow rapidly effectively shortening management cycles (Berkowitz et al., 1995). Specifically, mechanical management cycles along Yukon Territory transmission ROWs have decreased from ten to seven years (Shannon Mallory, Environmental Coordinator, Yukon Energy Corporation, pers. comm.). Thus, management strategies that may increase long-term effectiveness were examined. Herbicide application, the most common control technique, was examined because it can promote low growing shrub communities reducing and/or eliminating the need for large scale vegetation management (Niering and Goodwin, 1974; Bramble et al., 1991; Nowak and Ballard, 2005a). Within chemical control, there are different application methods that can have an impact on the effectiveness and the chemical load added to the

environment. Broadcast spray applications are often used initially; then, as IVM proceeds strategies adapt to more targeted approaches including cut stump and point injection (Nowak and Ballard, 2005a; b). However, prior to implementation, research into the different management techniques in the Yukon Territory needed to be investigated.

In northern latitudes, triclopyr and imazapyr have longer residence times in soils compared with temperate regions. Newton et al. (2008) reported the dissipation of triclopyr and imazapyr from Alaskan soils to occur rapidly during the growing season, slowing noticeably in the winter. In Sweden and Alaska, triclopyr residues were present up to two years after application, with the longest residence time in more northern regions (Torstenssen and Stark, 1982; Newton et al., 2008). In northern Ontario triclopyr residues were present at concentrations below 10% of the application rate 48 weeks after treatment (Stephenson et al., 1990). Imazapyr has been reported to have half-lives of 67 to 144 days after treatment along railway embankments in Sweden with residues detected up to 456 days after treatment in Alaska (Börjesson et al., 2004; Newton et al., 2008). To our knowledge, no laboratory toxicity assays with soil dwelling organisms have been published for triclopyr or imazapyr. However, Stephenson et al. (1990) hypothesized that triclopyr residues below 0.055 mg kg^{-1} would pose likely pose little unacceptable risk to the ecological community.

Predictive ecological risk assessments evaluate potential environmental impacts of a chemical or stressor to determine application methods that pose the least risk to the soil ecosystem (Suter, 2006). Further, deterministic approaches pair the results of standardized toxicity tests with real world concentrations to characterize the risks associated with the use of the chemical (CCME, 1996; Jansch et al., 2006; Suter, 2006). The objectives of this research were to evaluate the dissipation of triclopyr and imazapyr in northern latitude soils, as well as the associated toxicity to the soil invertebrate community. The final step links field dissipation (Chapter 2) to the standardized laboratory toxicity tests (Chapter 3), allowing for an estimate of the risk associated with adding herbicide application to the management regime for transmission ROWs in the Yukon Territory. Utilizing both predictive and deterministic approaches should allow for a systematic approach for the development of environmentally sound IVM programs.

4.2. PRINCIPAL FINDINGS

4.2.1. Field Dissipation

The dissipation of Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr) was assessed at five sites representing the main ecoregions where ROWs exist in the Yukon Territory. Specifically, the dissipation rates from a soil (LS) representative of Yukon Territory ROWs were determined and compared to herbicide concentrations at four additional sites to assess variation across ROW soils that have different soil properties. At the LS site, triclopyr from backpack spray application followed a biphasic distribution with 50% dissipation of the initial concentration within the first phase ($DT_{50\ BIPHASIC}$) occurring 1 DAT, whereas imazapyr dissipation followed first order kinetics with a DT_{50} of 16 DAT. Overall, it was expected that these herbicides would degrade slower in northern latitudes due to the colder climate that slows microbial activity in the soil. However, our results indicate a faster dissipation rate than those observed in other northern climates with results more comparable to those found in soils near Pueblo, Colorado and north central Colorado with half-lives ranging from 5-16 days and 82-286 days for triclopyr and imazapyr, respectively (Douglass et al., 2016b).

Average triclopyr residues at site LS were 1.17 and 0.42 mg a.i. kg d.w.$^{-1}$ at 1 and 30 DAT, respectively. However, the results from the LS soil should be interpreted with caution as 9.6 mm of precipitation fell within 48 hours after application (Environment Canada, 2015), increasing the potential for leaching. Higher concentrations were found in the other site soils (CAR, DAW, HJ1, HJ2). However, only the HJ2 site soil had significantly higher residues, possibly due to higher total organic carbon and clay contents (Table 2.2). Increased colloidal surfaces from the organic matter and clay contents may have increased adsorption of the herbicide particles resulting in the longer residence time in the HJ2 soil. Additionally, the LS soil had a lower organic matter and clay contents and higher sand content possibly resulting in higher leaching potential. Imazapyr dissipation showed no significant site differences indicating that imazapyr dissipation may not be significantly influenced by factors other than organic matter and clay content. While it is hypothesized that soil properties were playing a role in the dissipation of these herbicides, no soil property solely explained differences in dissipation rates.

Additionally, three treatment methods were assessed to determine how dissipation rates may differ across application techniques. Overall, backpack spray applications had the longest

dissipation times. The long residence time is expected since spray treatments have broad coverage, whereas the cut stump and point injection treatments are targeted application methods with herbicides applied directly to vegetative stems. Residues from the cut stump and point injection treatments dissipated considerably between 30 and 365 DAT for both herbicides. For cut stump application, triclopyr concentration was minimal or below detection limits, which was to be expected since root exudation is negligible (Braverman, 1995; Wahlers et al., 1997). Similarly, most imazapyr residues for point injection decreased between 30 and 365 DAT. However, concentrations for the point injection techniques were comparable to those for the backpack spray application at 30 and 365 DAT and were present at concentrations high enough to impact the growth of adjacent non-target species found in the treatment plot area (Isbister, 2016).

4.2.2. *Laboratory Soil Toxicity Tests*

The toxicity of Garlon XRT (triclopyr) and Arsenal Powerline (imazapyr) was assessed to obtain a greater understanding of the risk to soil dwelling organisms. Specifically, standardized toxicity tests were conducted using three soil invertebrates (*Enchytreaus crypticus*, *Folsomia candida*, and *Oppia nitens*) and three soil enzymes (phosphatase, arylsulfatase and B-glucosidase). The objective of the study was to determine the concentration of triclopyr and imazapyr residues that would affect 25% of the soil ecological community found in the organic layer from five sites representative of Yukon Territory ROWs. Concentrations expected from the calculated maximum field application rates were below the 28-d LC_{25} and 28-d EC_{25} for all species tested. Even at low thresholds, such as 28-d LC_{10} and 28-d EC_{10}, effects were generally not observed below concentrations expected from the maximum field application rates. While toxicity of triclopyr and imazapyr to the soil organisms tested appeared minimal, clear site differences in sensitivity were observed. It was hypothesized that soil pH and total organic carbon content influenced sorption of the herbicides to the soil colloids influencing bioavailability.

The surfactant, Hasten, was used for the imazapyr backpack spray mix and as such a surfactant control was added to examine the sensitivity of the soil invertebrates to this surfactant. Hasten had no effect on the survival or reproduction of *O. nitens,* but significant effects ($p<0.05$) were observed for *E. crypticus* and *F. candida*. The greatest reduction in survival and reproduction due to Hasten was observed in the DAW soil. Significant decreases in reproduction and survival of *E. crypticus* and *F. candida* in the DAW soil could be due to the surfactant alone, or the surfactant

could be acting in conjunction with the low soil pH to increase the sensitivity of this species to the herbicide. More studies are needed to further address the role of soil properties in the toxicity of the surfactant.

4.3. Linking Field Dissipation to Laboratory Toxicity Tests

Incorporating herbicide application into the vegetation management scheme along transmission ROWs in the Yukon Territory could improve long-term management by reducing environmental risk and decreasing management costs. This research examined herbicide dissipation in soil and the toxicity of triclopyr and imazapyr to soil invertebrates in order to close knowledge gaps associated with herbicide use in the Yukon Territory. A primary objective of the research was to examine the environmental risks to the soil community associated with adding herbicide application to the Yukon Energy Corporation's vegetation management strategy. Here risk is characterized by compiling environmentally relevant residue concentrations for both herbicides, as well as the effective concentrations values for different soil organisms; the results of which are briefly summarized above. Here, risk is characterized using two approaches: weight of evidence as per Environment Canada procedures and toxic exposure ratios utilized by the European Union (Environment Canada, 2007a; EC directive No 91/414 Annex VI (1991); EC Regulation No 1107/2009).

4.3.1. Weight of Evidence

Weight of Evidence (WOE) approaches examine the strength and weaknesses of a study using professional judgement to characterize the risks associated with a herbicide's usage (Environment Canada, 2007a; Rhomberg, 2015). WOE approaches are often used in environmental risk assessments to integrate different Lines of Evidence (LOE) often combining environmental fate data with toxicity data (Weed, 2005; Critto et al., 2007; Environment Canada, 2007a; Morales-Caselles et al., 2008; Semenzin et al., 2008; Rhomberg, 2015). Here, a WOE approach is used to evaluate the LOEs presented in Chapters 2 and 3. Specifically, the approach will combine the field dissipation data with laboratory toxicity test data to characterize the risks associated with adding herbicide application to vegetation management programs in the Yukon Territory. Not only will this link the field dissipation and laboratory toxicity data, but it will be an effective basis to communicate the ecological risks to stakeholders (Critto et al., 2007). Typically, Environment Canada uses the 28-d EC25 values for WOE approaches. Here, both the 28-d EC_{25}

and EC_{10} values were utilized. The 28-d EC_{10} value was used to allow for comparison to the quantitative TER approach which uses an EC_{10} toxicity estimate (EC directive No 91/414 Annex VI (1991); EC Regulation No 1107/2009, 2009).

4.3.1.1. Triclopyr

Triclopyr dissipation from the LS soil was biphasic with a $DT_{50\,BIPHASIC}$ of 1 DAT followed by a persistent phase. The persistent phase had a stable concentration of 0.52 mg a.i. kg d.w.$^{-1}$. The 28-day EC_{25} values, from the laboratory toxicity tests were all well above the triclopyr residue concentration quantified in the persistent phase. Further, the most sensitive endpoint calculated for the LS soil, the 28-d EC_{10} for *F. candida* (34 ± 9.97 mg a.i. kg d.w.$^{-1}$), was not only above the residue concentration in the persistent phase, but was also above the mean initial residue concentration (2.03 mg a.i. kg d.w.$^{-1}$) and the highest residue concentration (9.6 mg a.i. kg d.w.$^{-1}$) quantified in the field dissipation study for backpack spray application at the LS site (Figure 4.1). The 28-d EC_{10} for *F. candida* did, however, fall below the concentration expected when Garlon XRT is applied at the highest recommended application rate for the LS site (41.3 mg a.i. kg d.w.$^{-1}$).

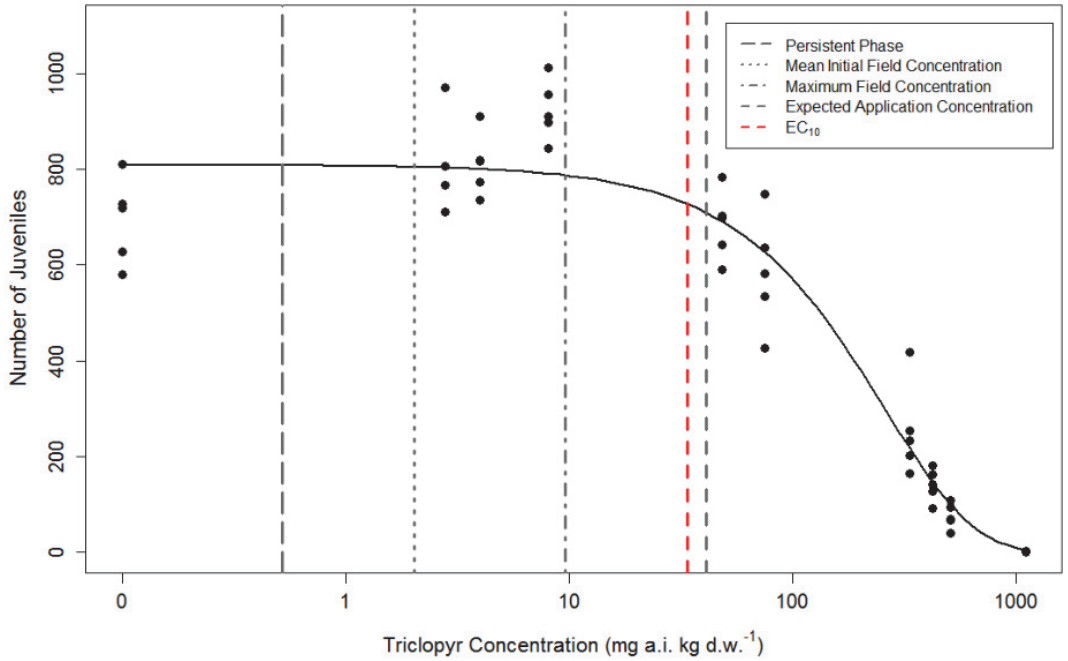

Figure 4.1. Dose-response curve for *F. candida* reproduction from the toxicity test conducted with LS soil linked with field dissipation data from the LS site for triclopyr. The black dots represent individual data points with the black line representing the dose-response curve. The vertical grey lines represent environmental concentrations quantified from the dissipation study. The long-dashed line represents the concentration of the persistent phase obtained from the biphasic distribution (0.52 mg a.i. kg d.w.$^{-1}$), the dotted line represents the mean initial concentration from the LS site (2.03 mg a.i. kg d.w.$^{-1}$) and dot-dash line represents the maximum residue concentration quantified from the backpack spray treatment at the LS site (9.6 mg a.i. kg d.w.$^{-1}$) and the small dash line represents the expected application concentration (41.3 mg a.i. kg d.w.$^{-1}$). The red dashed line represents the *F. candida* 28-d EC_{10} value (34.0 mg a.i. kg d.w.$^{-1}$).

While dissipation was quick within the LS soil, the other four soils appeared to have slower dissipation. The lowest 28-d EC_{10} value (*E. crypticus* in the CAR soil) was above the concentration quantified 1 DAT (11 mg a.i. kg d.w.$^{-1}$) and 30 DAT (4.1 mg a.i. kg d.w.$^{-1}$) from the backpack spray application at the CAR site (Figure 4.2). Furthermore, the lowest 28-d EC_{10} calculated for triclopyr was above the maximum residue concentration obtained from the cut stump/point injection treatments (1.34 mg a.i. kg d.w.$^{-1}$) but below the concentration expected when Garlon XRT is applied at the highest recommended application rate for the CAR site (75.5 mg a.i. kg d.w.$^{-1}$) The results of the WOE for triclopyr indicate that triclopyr dissipates rapidly in soil ($DT_{50\ BIPHASIC}$ of 1 DAT) and has minimal effects on soil ecological communities at normal application rates. However, the application rate could be adjusted to reduce potential risk associated with the highest recommended application rate.

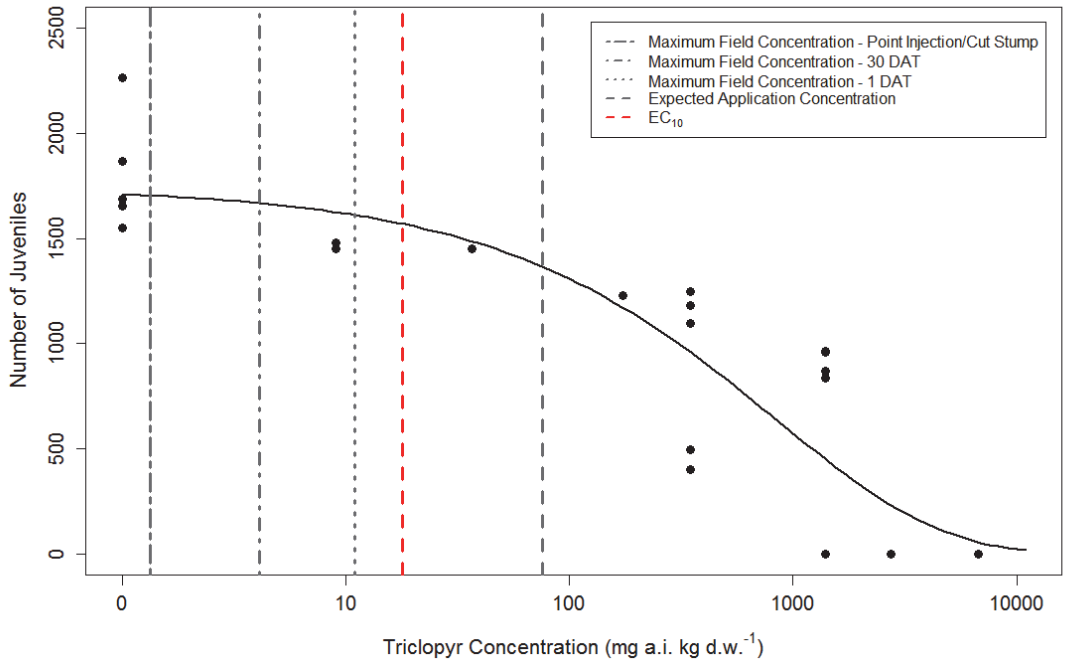

Figure 4.2. Dose-response curve for the most sensitive species and endpoint for triclopyr, *E. crypticus* reproduction, linked with site specific field dissipation data from the CAR site. The black dots represent individual data points with the black line representing the dose-response curve. The vertical grey lines represent environmental concentrations quantified from the dissipation study. The two dash line represents the maximum concentration observed in the point injection/cut stump treatments (1.34 mg a.i. kg d.w.$^{-1}$), the dot-dash line represents the highest concentration observed 30 DAT (4.1 mg a.i. kg d.w.$^{-1}$), the dotted line represents the highest concentration observed 1 DAT (11 mg a.i. kg d.w.$^{-1}$) and the small dash line represents the expected application concentration (75.5 mg a.i. kg d.w.$^{-1}$). The red dashed line represents the *E. crypticus* 28-d EC_{10} endpoint value (18.0 mg a.i. kg d.w.$^{-1}$).

4.3.1.2. Imazapyr

Imazapyr dissipation from the backpack spray treatment in the LS soil followed first order kinetics with a DT_{50} of approximately 16 DAT. Mean residue concentrations (0.16 mg a.i. kg d.w.$^{-1}$) observed at 0 DAT at the LS site were below the 28-d LC_{25} and 28-d EC_{25} values obtained from the imazapyr toxicity tests with all soil organisms evaluated (Figure 4.3). Further, the 28-d EC_{10} value was above the highest field concentration (0.37 mg a.i. kg d.w.$^{-1}$) quantified for the backpack spray application and the expected application concentration (6.56 mg a.i. kg d.w.$^{-1}$) at the LS site.

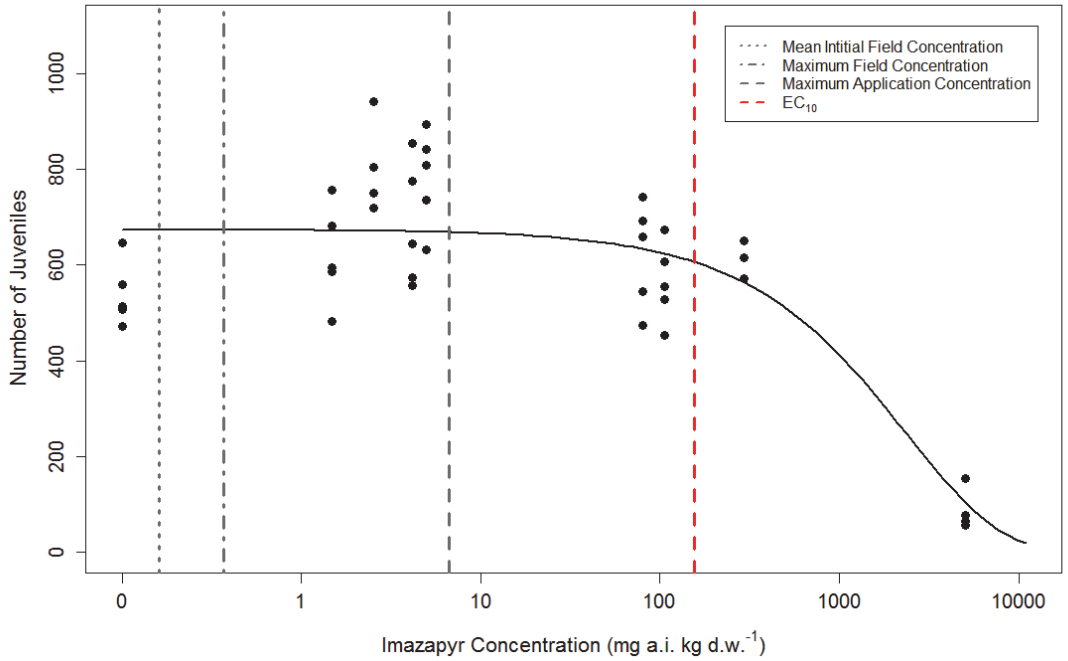

Figure 4.3. Dose-response curve for *F. candida* reproduction in LS soil linked with field dissipation data from the LS site for imazapyr. The black dots represent individual data points with the black line representing the dose response curve. The vertical grey lines represent environmental concentrations quantified from the dissipation study. The dotdash line represents highest overall concentration (1.34 mg a.i. kg d.w.$^{-1}$), the dotted line represents the mean initial concentration (0.16 mg a.i. kg d.w.$^{-1}$) and the small dash line represents the expected application rate (6.56 mg a.i. kg d.w.$^{-1}$). The red dashed line represents the *F. candida* 28-d EC_{10} endpoint value (156 mg a.i. kg d.w.$^{-1}$).

Dissipation at the LS site appeared to occur faster when compared to residue concentrations at the four other sites. The lowest 28-d EC_{10} (23.3 mg a.i. kg d.w.$^{-1}$) for imazapyr was obtained from the *E. crypticus* tests using the DAW soil. Residue concentrations obtained 1 DAT (0.079 mg a.i. kg d.w.$^{-1}$) and 30 DAT (0.30 mg a.i. kg d.w.$^{-1}$) from soil samples collected at the DAW site were well below the 28-d EC_{10} value (Figure 4.4). Additionally, the highest concentration obtained from the cut stump/point injection treatments was 0.57 mg a.i. kg d.w.$^{-1}$ and the expected application concentration (6.56 mg a.i. kg d.w.$^{-1}$) were below the 28-d EC_{10} value. The results of the WOE for imazapyr indicate that the herbicide dissipates gradually in soil but should not impact greater than 10% of the soil organisms tested and at normal application rates.

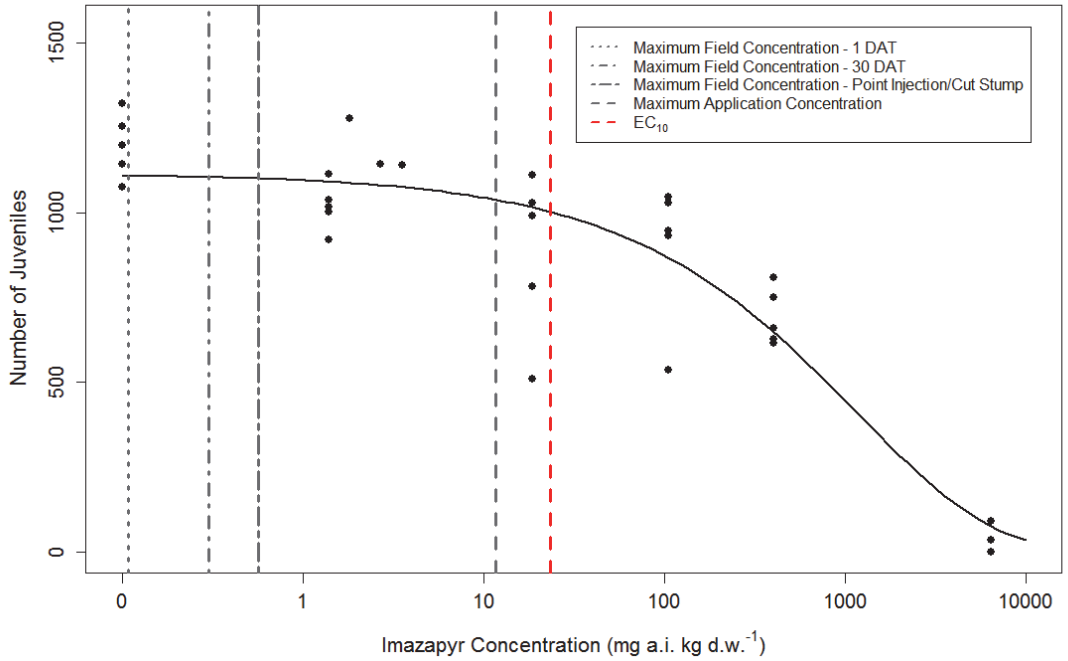

Figure 4.4. The lowest toxicity estimate, *E. crypticus* reproduction in the DAW soil, linked to the field dissipation data for imazapyr. The black dots represent individual data points from the toxicity test with the black line representing the dose response curve. The twodash line represents the maximum concentration observed in the point injection/cut stump treatments (0.57 mg a.i. kg d.w.$^{-1}$), the dotdash line represents the highest concentration observed 30 DAT (0.30 mg a.i. kg d.w.$^{-1}$) and the dotted line represents the highest concentration observed 1 DAT (0.079 mg a.i. kg d.w.$^{-1}$). The red dashed line represents the *E. crypticus* 28-d EC_{10} endpoint value (23.3 mg a.i. kg d.w.$^{-1}$).

While imazapyr appears to present no unacceptable risk to the soil invertebrates tested, the herbicide was highly toxic to non-target vegetation with impacts observed greater than two years after application (Isbister et al., 2017). Therefore, a small aging study was conducted using *F. candida* to determine if imazapyr toxicity increases over time (Appendix F). It was hypothesized that over time toxicity of imazapyr to soil invertebrates could increase. It is possibly that toxicity could occur for one of two reasons: the imazapyr metabolites are more toxic to soil invertebrates or since imazapyr is a chiral herbicide and one of the enantiomers could exhibit delayed toxicity. Therefore, *F. candida* was exposed to soils from 0 to 60 days after dosing with imazapyr. Decreases in reproduction were noted at 14 days after dosing in the LS soil. The lowest juvenile production observed in the LS soil occurred 30 days after dosing replicates. However, while not statistically significant, a decrease in juvenile production is noted starting 14 days after dosing (Figure F.2). Overall, the results of the small study indicated that imazapyr toxicity may increase over time but longer dosing intervals are needed to fully test the theory.

4.3.2. Toxic Exposure Ratio

Toxic Exposure Ratios (TER), also known as risk quotients, are used in ecological risk assessment to extrapolate standardized test results and exposure estimates to real world scenarios (Christl et al., 2016; Ernst et al., 2016). The TER calculation (Eq. 5.1) uses a the 28-d EC_{10}, and divides it by the Potential Environmental Concentration (PEC_{soil}) (Christl et al., 2016; Ernst et al., 2016):

$$Toxic\ Exposure\ Ratio\ = \frac{EC_{10}}{PEC_{soil}} \qquad \text{(Equation 4.1)}$$

The European Union uses a critical trigger value of 10 or below for acute/short term studies and 5 or below for chronic/long-term studies to account for uncertainties (EC directive No 91/414 Annex VI (1991); EC Regulation No 1107/2009). Therefore, the use of TER allows for a prediction of the environmental risk associated with a compound that is protective but not too conservative (Christl et al., 2016; Ernst et al., 2016).

The dissipation of triclopyr and imazapyr is relatively intermediate with calculated DT_{50} values of less than 28 days. Since dissipation is generally rapid and less than the length of the chronic invertebrate tests utilized (standardized 28-day toxicity tests), the TER approach is appropriate for estimating the environmental risk in Yukon Territory soils. Evidence provided in the previous chapters suggest that the soil properties are influencing the sensitivity of the organisms tested to both triclopyr and imazapyr. Therefore, the TER was calculated for each site soil based on the maximum PEC_{soil} concentrations quantified at 1 DAT, 30 DAT and 365 DAT from the backpack spray treatment, where data are available. At 1 DAT, TER values below the critical trigger value of 10 indicate that a higher-level assessment is required to adequately estimate environmental risk for acute exposure, whereas, calculated TER values below the chronic exposure trigger value of 5 at 30 and 365 DAT prompt additional assessments for chronic exposure (Ernst et al., 2016). The EC_{25} endpoints generated for each site soil, per Environment Canada protocols (Environment Canada, 2007a), were examined with the lowest EC_{25} value for each site soil used in the TER calculation. All TERs calculated using EC_{25} values were above critical trigger values, therefore, a more sensitive endpoint, 28-d EC_{10}, was used as per the European Union approach (Table 4.1) (EC directive No 91/414 Annex VI (1991),; EC Regulation No 1107/2009, 2009). TER values for 365 DAT are not provided because triclopyr residues were not quantified.

Table 4.1. Toxic Exposure Ratios (TER) for triclopyr and imazapyr calculated using the lowest 28-d EC_{10} endpoint generated for each site soil, the expected application concentration and the Potential Environmental Concentration (PEC_{soil})from the soil at 1, 30 and 365 days after backpack spray treatment. 28-d EC_{10} values were used to determine the TERs because all TERs calculated with 28-d EC_{25} values were above the critical trigger values. The acute TER values used PEC_{soil} values from 1 day after treatment and a critical trigger value of 10. The chronic TER values used PEC_{soil} values from 30 and 365 days after treatment and have a critical trigger value of 5. Bold and underlined font indicates TER values below the critical trigger value.

Herbicide	Site	28-d EC_{10}† (mg a.i. kg d.w.$^{-1}$)		Application Rate	1 DAT‡		30 DAT‡		365 DAT‡	
		Species	Value	Expected Concentration¶ (mg a.i. kg d.w.$^{-1}$)	TER	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER
Triclopyr	CAR	*E. crypticus*	18 ± 21.3	75.5	**0.24**	11	**1.64**	4.1	**4.39**	NA	NC
	DAW	*E. crypticus*	76 ± 17.8	73.5	**1.03**	2.9	26.2	16	**4.75**	NA	NC
	HJ1	*F. candida*	188 ± 74.3	45.9	**4.10**	35	**5.37**	4	47.0	NA	NC
	HJ2	*F. candida*	161 ± 135	34.2	**4.71**	11	14.6	10	16.1	NA	NC
	LS	*F. candida*	34 ± 9.91	41.3	**0.82**	2.3	14.8	0.55	61.8	NA	NC
Imazapyr	CAR	*E. crypticus*	392 ± 264	12.0	32.7	0.2	1960	0.16	2450	0.005	78400
	DAW	*E. crypticus*	23.3 ± 21.3	11.7	**1.99**	0.078	299	0.3	77.7	0.03	777
	HJ1	*E. crypticus*	176 ± 116	7.3	24.1	0.67	263	0.15	1173	0.027	6519
	HJ2	*F. candida*	213 ± 108	5.44	39.1	0.67	318	0.28	761	0.008	26625
	LS	*F. candida*	156 ± 129	6.56	23.8	0.1	1560	0.19	821	0.032	4875

† 28-d EC10: Effective Concentration that will limit reproduction by 10% based on a 28-day test

‡ DAT: Days After Treatment.

¶ Expected application concentration calculated using the recommended application rate (4530 g triclopyr ha^{-1} and 720 g imazapyr ha^{-1}, the bulk density for each site soil (Table 2.2) and a sample depth of 3 cm.

TER values below 5, the critical trigger value for chronic exposure, were only observed in the backpack spray treatment and for the most sensitive 28-d EC_{10} determined, TER values for both acute and chronic exposures were above critical triggers values for both cut stump and point injection treatments (Appendix G). For triclopyr, TER values for the expected application concentrations for triclopyr were all below the critical trigger value of 10 for acute exposure indicating that there may be some unacceptable risk through the use of triclopyr. However, using the PEC_{soil} values, the calculated TER value was only under the critical trigger value of 10 for acute exposure in the CAR and HJ1 soil. At 30 DAT, the calculated TER values for the CAR and DAW soils were below the chronic trigger value. Soils that are more acidic with higher TOC (>12%) may be at greater risk and a higher-level assessment (i.e. community level study) could be conducted to further evaluate the risks that triclopyr may pose if applied via broadcast application at these sites. For imazapyr, only the TER from the DAW soil using the expected application concentrations was below the critical trigger value. The remaining TER values calculated using PEC_{soil} values for imazapyr were above acute and chronic trigger values indicating, with the exception of DAW, residue concentrations quantified in the field study do not pose an unacceptable risk to the soil ecological community.

Overall, the TER approach is a more conservative approach than WOE because it includes an uncertainty factor, whereas, the WOE approach is based on estimating risk through professional expertise and judgement. Using the WOE approach, it was possible to demonstrate that PEC_{soil} concentrations were below not only the 28-d EC_{25}, but also the 28-d EC_{10} values in all cases. The TER approach identified that some ecological risk is present to soil organisms through the use of triclopyr with no identified risks through the application of imazapyr. However, with the exception of CAR and HJ1 at 1 DAT, the identified risks of triclopyr application are close to the critical trigger value and it is likely that communities would recover less than one year after application, if impacted at all. The WOE and TER methods presented above indicate that the herbicides, triclopyr and imazapyr, may be acceptable for use in the Yukon Territory due to relatively intermediate dissipation periods and low toxicity to the soil invertebrates tested.

Due to the intrinsic value of non-target vegetation to First Nations communities and other stakeholders, investigating the impacts of management techniques on non-target vegetation is vital to the development of an IVM program in the Yukon Territory. Using the IC_{50}, the inhibition

concentration causing greater than 50% seedling growth reduction, the effect of herbicide application on two non-target plant species, *Achillea millefoluim* and *Chamerion angustifolium*, was assessed (Isbister et al., 2017). The toxic effects were similar across the five sites with the lowest IC_{50} for triclopyr observed at 1.59 ± 0.47 mg a.i. kg $d.w^{-1}$ (Isbister et al., 2017). This concentration is above the persistent concentration quantified for triclopyr in the LS soil but is well below the maximum soil residue concentration of 11 mg a.i. kg $d.w^{-1}$ observed 1 DAT in the CAR soil. However, a damage assessment conducted 365 DAT found less than 10% damage to non-target forbs in triclopyr application plots (Isbister, 2016). IC_{50} estimates were not calculated for the imazapyr dose response tests because even at the lowest dose (2 mg a.i. kg $d.w.^{-1}$) there was greater than 75% inhibition (Isbister et al., 2017). A vegetative vigour test using foliar application spray indicated the IC_{50} estimates were at 1.5% of the maximum application rate for both species, examined (Isbister et al., 2017). Furthermore, damage assessments from imazapyr treatment plots conducted showed 25-35% damage to non-target forbs 365 DAT (Isbister, 2016). Overall, non-target vegetation in the triclopyr treatment plots recovered within 365 DAT, but there was significant damage to non-targets within imazapyr treatment plots (Isbister, 2016). Based on this evidence and the data presented here, triclopyr may be a better choice for implementation along Yukon Territory ROWs.

4.4. Implications for Herbicide Application in Northern Climates

Integrated Vegetation Management (IVM) practices are proposed for use in the Yukon Territory where transmission ROWs are currently managed solely through mechanical techniques. Mechanical techniques, such as brushing and mowing, promote rapid woody deciduous growth leading to shortened management cycles (Nickerson, 1992; Nowak and Ballard, 2005a). Utilizing IVM could allow for adaptive management practices that merge sound science with appropriate community engagement. This could ultimately lead to more effective vegetation management practices in the Yukon Territory. This thesis examined the dissipation and toxicity of triclopyr and imazapyr in soils to provide an estimate of ecological risk pertaining to herbicide use along Yukon Territory ROWs. The WOE and TER methods presented above indicate that the herbicides, triclopyr and imazapyr, may be acceptable for use in northern latitudes due to intermediate dissipation periods and low toxicity to the soil invertebrates tested. However, soil invertebrates are only one element of the ecological community and to fully evaluate risk all components of the

ecosystem should be considered, including non-target vegetation. The results of the non-target vegetation tests indicate that triclopyr is the better choice for implementation along ROWs in the Yukon Territory due to an IC_{50} value above the persistent phase and minimal damage 365 DAT (Isbister et al., 2017).

The objective of this research was to determine the efficacy and environmental impact of various vegetation control techniques for ROWs in the Yukon Territory, with a focus on chemical management techniques. Utilizing all lines of evidence including the data presented in Chapters 2 and 3 of this thesis, as well as vegetation studies conducted by Isbister (2016) triclopyr appears to present less potential risk overall. This hypothesis is based on rapid initial dissipation ($DT_{50\ BIPHASIC}$ of 1 DAT), low risk to soil invertebrates, and non-target vegetative recovery within one growing season.

4.5. Future Directions

Overall, triclopyr presents less potential risk to the environment than imazapyr based on the lines of evidence discussed above and may be the better option for implementation. While one specific soil characteristic could not be identified, it is evident that soil properties are linked to herbicide dissipation along these ROWs. It is suggested that additional work using northern soils, under controlled climatic conditions typical of a Yukon year, would better delineate the role of soil properties (ie. pH, soil organic matter, texture and cation exchange capacity) in the dissipation of herbicides in northern Boreal forest soils. Further, increasing the number of soils assessed would increase confidence in the herbicide persistence results and would also allow for a greater understanding of the soil ecological community along Yukon Territory ROWs.

For sites, such as CAR and DAW, where soil properties, including pH, texture and organic matter content, appear to have influenced the toxicity of herbicides to the soil organisms tested, a community level assessment could be conducted. Conducting a community level assessment at these sites would allow for an increased understanding of the indirect effects herbicide application may have on soil invertebrate communities. These indirect effects could include altered populations dynamics, altered forage quality, changes in biodiversity and/or alterations to predator-prey dynamics (Freemark and Boutin, 1995). Increasing knowledge of indirect effects associated with herbicide application could increase understanding of the larger soil ecosystem impacts in northern latitudes.

The research presented in this thesis indicates that herbicide use is an alternative option for vegetation management along transmission ROWs in the Yukon Territory. However, public consultations are needed to effectively communicate IVM principles and research while being sensitive to the fears and opinions of the public (Lahr and Kooistra, 2010). While triclopyr and imazapyr dissipated rapidly with DT_{50} values of 1 and 16 DAT, respectively, low level concentrations persisted in soil more than 365 DAT. However, herbicide residues were below concentrations that adversely affect more than 25% of the soil invertebrate community. Linking herbicide dissipation to soil ecological risk indicates that both triclopyr and imazapyr are good candidates for use along Yukon Territory ROWs. Yet, when all lines of evidence, including non-target vegetation, are utilized to characterize the risk associated with herbicide application, triclopyr, due to its relatively rapid dissipation, low toxicity to soil invertebrates, and the non-target vegetation species recovery within one growing season, may be more appropriate than imazapyr for use along ROWs in the Yukon Territory. The next step involves adequately communicating these results to the public.

Different vegetation communities and soil properties have the ability to influence the dissipation and toxicity of herbicides. Thus, developing Geographic Information System (GIS) layers that provide local data on vegetation communities and soil properties with past vegetation control measures could help to effectively monitor treatment efficacy over time and assess risk to rapidly identify appropriate adaptive management techniques. A layer identifying different soil properties could help determine where herbicide application may not be appropriate including soils with higher leaching potential such as soils with limited OM and high sand content. Further, integrative GIS programs allow for a visual way to communicate components of an IVM program to the public such as where different application techniques should be employed to reduce residence time in soil or impacts on non-target vegetation. It would also aid in identifying areas, such as water bodies and private property, where caution should be taken when selecting a management method (Lahr and Kooistra, 2010).

The WOE and TER methods described above can also be employed to communicate ecological risk to the public. The WOE method is qualitative method of characterizing risk, which utilizes professional judgment to determine the risk level. For example, Figure 4.1 shows an effective way to present WOE utilizing research data as a visual aid to communicate minimal ecological risk

associated with actual environmental concentrations. Specifically, Figure 4.1 shows that the environmental concentrations of triclopyr in the LS soil are below the most sensitive endpoint derived from the toxicity tests. Figures 4.2, 4.3 and 4.4 show similar results indicating no risk is associated with herbicide application in northern soil. On the other hand, TERs provide a quantitative approach with a trigger value allowing for a simple way to communicate the risks of herbicide application. For example, some TERs calculated using PEC_{soil} for triclopyr were below the trigger value at 1 and 30 DAT. However, in all but one case, the 30 DAT TERs were close to the trigger value indicating minimal risk to the soil ecological community with a high probability of recovery within one year. Utilizing GIS mapping, as well as, WOE and TER techniques will allow for transparency and effective communication as part of an IVM program.

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APPENDIX A: METHODS FOR DETERMINATION OF SOIL CHARACTERISTICS

Modified from:

Isbister, K.M. 2016. 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. MSc. Thesis. University of Saskatchewan, Saskatoon, SK.

List of Tables

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 the 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 105ºC for 24 hours
11. Weigh dried soil to nearest mg
12. Calculate bulk density (g/cm^3) with the equation:

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

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

Determination of pH

1. Sieve each soil sample to 2 mm
2. Weigh out five replicates of 4 ± 0.05 g sub-samples unto glass test tubes
3. Add 20 mL of 0.01M $CaCO_2$ solution to each sample and apply lids to test tubes
4. Shake for 30 minutes
5. Let site 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. Joesph, MI).

Sample Preparation:
1. Air dry soil samples for 48 hours
2. Grind each soil sample to very fine 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, Mississauga, ON) for each soil sample
4. Weigh 200 ± 10 mg 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 three 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-632 (LECO Corp, St. Joseph, MI).

Sample Preparation:
1. Air dry soil samples for 48 hours
2. Grind each soil sample to a very fine powder with Rusch ZM200 plant grinder at 14, 000 RPM
3. Use a 3 g subsample to determinepercent moisture in Mettler Toledo MJ33 Moisture Analyzer (Mettler Toledo Canada, Mississauga, ON) for each soil sample
4. Weigh 200 ± 10 mg 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 desiccator 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 105ºC overnight to remove residual moisture and HCl

Analysis (LECO-632):

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

Calculations:

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

Simplified Particle Size Analysis Using Fleakers

From: Indorante, SJ, Follmer, LR, Hammer, Koenig, PG. 1990. Particle size analysis by a modified pipette procedure. Soil Science Society of America Journal 54: 560-563.

Day One:

1. Sample weighing
   1) Weigh 10 ± 0.1g of air-dried, <2 mm soil to three decimal placed; record sample name (and any comments), weight, and Erlenmeyer flask number
   2) Place sample into Erlenmeyer flask
2. Pre-treatment to remove organic matter:
   Use this pre-treatment if sample is from an A horizon (Ah, Ap, Ahe) or if soil organic carbon > 1%. Otherwise proceed to step 11.
   1) Fill a pan with cold water and place near hot plate
   2) Put sand-filled tray on hot plate and turn dial to position 6
   3) Add ~10 mL of distilled water to Erlenmeyer flask
   4) Add 10 mL of H_2O_2 to Erlenmeyer flask, stir and cover with watch glass
   5) Observe closely for several minutes. If excessive frothing occurs (ie. frothing to top of Erlenmeyer flask), cool the container in cold water. If excessive frothing continues transfer sample to larger beaker (eg. 1000 mL)
   6) When frothing subsides, heat contents of Erlenmeyer flask to 90ºC. Watch for frothing.
   7) Once temperature is reached, continue adding 5 mL of H_2O_2 until most organic matter is removed (as observed by colour of sample (*colour should become grey with time*) and rate of reaction). *Usually only 1 or at most 2 additions are required. Rinse down sides of flask occasionally*
   8) Continue heating the sample for about 45 minutes after the final addition of H_2O_2 to remove any excess H_2O_2
   9) Please Erlenmeyer flask in oven at 105ºC and dry overnight; weigh Erlenmeyer flask and treated sample next day

Day Two:

3. Pre-treatment to disaggregate soil:
    1) Add 10 mL of 10% sodium hexametaphosphate solution
    2) Bring to approximately 150 mL with distilled water
    3) Fill one Erlenmeyer with only water and 10% sodium hexametaphosphate for correction
    4) Stopper tightly
    5) Place flasks on end-over-end shaker (*securely, but do not overtighten or flasks will break*) and shake overnight

Day Three:

4. Pipette Analysis
    1) Transfer contents of Erlenmeyer flasks into Fleakers *(ensuring that the correct sequence of samples is followed)*. Include contents from the sodium hexametaphosphate only flask
    2) Bring Fleakers to exactly 400 mL using room-temperature distilled water. Cover each Fleaker with a watch glass.
    3) Determine temperature of suspensions in Fleakers. Use this to determine when the clay aliquot will be taken following Table A.1:

Table A.1. Settling times at corresponding temperatures for pipetting <2 μm fraction at a 5 cm depth.

Temperature (°C)	Settling Times (h:min)	Temperature (°C)	Settling Times (h:min)
17	4:22	24	3:41
18	4:15	25	3:36
19	4:09	26	3:31
20	4:03	27	3:26
21	3:57	28	3:22
22	3:51	29	3:17
23	3:46	30	3:13

    4) Record the time. Start 2-minute timer on watch.
    5) After 1:30 has elapsed remove watch glass and from first Fleaker, cap with a rubber stopper and shake vigorously until 2-minute timer sounds on watch
    6) Rinse soil from stopper into Fleaker and replace watch glass
    7) Start to shake second Fleaker after 1:30 has elapsed. Continue shaking at 2 minute intervals until all Fleakers have been shaken
    8) After the appropriate time period from table 1 has elapsed, start the 2-minute timer on a watch and pipette the clay fraction from a 5 cm depth with a 10 mL pipette
    9) Discharge the aliquot into a tared 50 mL beaker and rinse pipette
    10) Take an aliquot from the Fleaker containing only the sodium hexametaphosphate and use as a correction factor
    11) When all clay aliquots have been taken, place a 50 μm sieve and funnel on top of 1000 mL settling column
    12) Swirl sample around in Fleaker to re-suspend soil at bottom of the Fleaker

13) Slowly empty contents of the Fleaker into the sieve. *If the sieve begins to fill with water, direct a jet of distilled water from a squeeze bottle onto the mesh to facilitate water passage through the mesh*
14) Use a jet of water from squeeze bottle to remove all remaining soil from the Fleaker into the sieve
15) Use jet of water to wash soil on sieve until water emerging from underside of sieve is clear. *Make sure not to fill cylinder past the 1000 mL mark.*
16) Use jet of water to transfer soil from sieve into a tared 50 mL beaker. *Ensure number on beaker matches number of Fleaker*
17) Place all beakers in oven at 105ºC and dry overnight
18) Weigh sample and beaker
19) Drain all columns into large pail and add 100 mL of 1M $CaCl_2$ and 10 mL of 1M HCl to speed flocculation

Determination of Gravimetric Soil Moisture

Modified from *Methods in Applied Soil Microbiology and Biochemistry, 1995, p. 105*

1. Preheat soil designated drying oven to 105˚C.
2. Label & Weigh tin tray, record exact weight.
3. Tare the scale to 0 and add 10g soil in even layer to tin tray. Record weight.
4. When samples are ready, load evenly into oven, avoiding the temperature extreme of the bottom shelf.
5. Leave samples to dry for 24 plus and record exact weight of soil & tin
6. Calculations:

$$\frac{\text{Mass water}}{\text{Mass oven dry soil}} \times 100$$

Mass water = wet soil weight – ((tin + soil weight post drying) - tin)
Mass oven dry soil = (tin + soil weight post drying) - tin

APPENDIX B: NOMINAL AND MEASURED CONCENTRATIONS FOR DOSE LEVELS USED FOR THE INVERTEBRATE TOXICITY TESTS

List of Tables

Table B.1. Nominal and measured concentrations for dose levels used to test the toxicity of triclopyr to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Sample ID's including rep 1, rep 2 and rep 3 were dosed and analyzed in triplicate to assess consistency of dosing. The symbol † was used to identify concentrations that were not consistent with adjacent dose levels and were omitted as a result. Dose levels that were omitted were estimated using adjacent dose levels.

Table B.2. Nominal and measured concentrations for dose levels used to test the toxicity of imazapyr to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Sample ID's including rep 1, rep 2 and rep 3 were dosed and analyzed in triplicate to assess consistency of dosing. The symbol † was used to identify concentrations that were not consistent with adjacent dose levels and were omitted as a result. Dose levels that were omitted were estimated using adjacent dose levels.

Table B.1. Nominal and measured concentrations for dose levels used to test the toxicity of triclopyr to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Sample ID's including rep 1, rep 2 and rep 3 were dosed and analyzed in triplicate to assess consistency of dosing. The symbol † was used to identify concentrations that were not consistent with adjacent dose levels and were omitted as a result. Dose levels that were omitted were estimated using adjacent dose levels.

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg $d.w.^{-1}$)
TX1	CAR - G0.25X - rep 1	90.5	19	9.1	10.1
TX2	CAR - G0.25X - rep 2	94.3	19	8.2	8.70
TX3	CAR - G0.25X - rep 3	92.0	19	7.6	8.27
TX4	CAR - G0.5X	90.8	38	13	14.3
TX5	CAR - G1X	92.4	75	34	36.8
TX6	CAR - G5X	92.6	377	160	173
TX7	CAR - G10X	92.2	755	320	347
TX8	CAR - G50X	121.5	3775	1700	1399
TX9	CAR - G100X	117.3	7550	3200	2728
TX10	CAR - G250X - rep 1	50.3	18874	5400	10735
TX11	CAR - G250X - rep 2	111.3	18874	5100	4582
TX12	CAR - G250X - rep 3	115.3	18874	5600	4856
TX39	DAW - G0.25X - rep 1	91.6	18	8.6	9.39
TX40	DAW - G0.25X - rep 2	93.1	18	8.6	9.24
TX41	DAW - G0.25X - rep 3	88.9	18	4.5	5.06
TX42	DAW - G0.5X	93.5	37	9.8	10.5
TX43†	DAW - G1X	111.2	73	12	10.8
TX44	DAW - G5X	92.0	367	29	31.5
TX45	DAW - G10X - rep 1	82.9	735	99	119
TX46	DAW - G10X - rep 2	94.2	735	130	138
TX47†	DAW - G10X - rep 3	93.5	735	23	24.6
TX48	DAW - G50X	95.7	3675	370	387

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg d.w.$^{-1}$)
TX49	DAW - G100X - rep 1	91.9	7350	1000	1089
TX50	DAW - G100X - rep 2	92.7	7350	790	852
TX51	DAW - G100X - rep 3	90.9	7350	950	1045
TX52	DAW - G250X	88.0	18250	1400	1591
TX13	HJ1 - G0.25X	62.3	11	6.4	10.3
TX14	HJ1 - G0.5X - rep 1	65.0	23	13	20.0
TX15	HJ1 - G0.5X - rep 2	65.1	23	13	20.0
TX16	HJ1 - G0.5X - rep 3	63.3	23	8	12.6
TX17	HJ1 - G1X	65.2	46	15	23.0
TX18	HJ1 - G5X - rep 1	64.5	230	81	126
TX19	HJ1 - G5X - rep 2	63.0	230	85	135
TX20	HJ1 - G5X - rep 3	63.8	230	160	251
TX21	HJ1 - G10X	65.6	459	160	244
TX22	HJ1 - G50X	64.2	2296	700	1090
TX23	HJ1 - G100X - rep 1	62.3	4593	950	1524
TX24	HJ1 - G100X - rep 2	61.1	4593	1000	1637
TX25	HJ1 - G100X - rep 3	60.8	4593	1200	1975
TX26	HJ1 - G250X	64.0	11481	1600	2502
TX27	HJ2 - G0.25X	50.6	9	17	33.6
TX28	HJ2 - G0.5X	55.9	17	14	25.0
TX29[†]	HJ2 - G1X - rep 1	55.8	34	13	23.3
TX30[†]	HJ2 - G1X - rep 2	54.6	34	27	49.4
TX31	HJ2 - G1X - rep 3	55.1	34	34	61.7
TX32	HJ2 - G5X	55.1	171	86	156
TX33	HJ2 - G10X	56.1	342	140	249
TX34	HJ2 - G50X - rep 1	54.3	1712	890	1638

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg $d.w.^{-1}$)
TX35	HJ2 - G50X - rep 2	60.7	1712	1000	1647
TX36	HJ2 - G50X - rep 3	55.8	1712	1100	1973
TX37	HJ2 - G100X	55.4	2568	1300	2347
TX38	HJ2 - G250X	50.9	17121	2300	4518
TX53	LS - G0.25X	96.1	10	2.7	2.81
TX54	LS - G0.5X - rep 1	96.5	21	4.1	4.25
TX55	LS - G0.5X - rep 2	97.6	21	3.7	3.79
TX56	LS - G0.5X - rep 3	97.7	21	3.8	3.89
TX57	LS - G1X	97.6	41	7.9	8.09
TX58	LS - G5X	96.9	206	47	48.5
TX59	LS - G10X	111.3	413	84	75.5
TX60	LS - G50X	95.0	2064	320	337
TX61	LS - G100X	97.3	4127	500	514
TX62	LS - G250X - rep 1	92.4	10318	1100	1190
TX63	LS - G250X - rep 2	98.8	10318	1000	1012
TX64	LS - G250X - rep 3	96.4	10318	1100	1141

† Measured concentration omitted because not consistent with adjacent concentration values

Table B.2. Nominal and measured concentrations for dose levels used to test the toxicity of imazapyr to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Sample ID's including rep 1, rep 2 and rep 3 were dosed and analyzed in triplicate to assess consistency of dosing. The symbol † was used to identify concentrations that were not consistent with adjacent dose levels and were omitted as a result. Dose levels that were omitted were estimated using adjacent dose levels.

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg $d.w.^{-1}$)
TX66	CAR - A0.2X - rep 1	173.1	2.4	2.4	1.39
TX67	CAR - A0.2X - rep 2	175.0	2.4	2.2	1.26
TX68	CAR - A0.2X - rep 3	178.4	2.4	3.8	2.13
TX69	CAR - A0.4X	174.0	4.8	4.7	2.70
TX70	CAR - A0.8X	174.7	9.6	5.4	3.09
TX71	CAR - A1X	176.6	12	8.8	4.98
TX72	CAR - A10X	172.3	120	57	33.1
TX73	CAR - A50X	173.0	600	270	156
TX74	CAR - A150X	169.8	1800	590	347
TX75	CAR - A2000X - rep 1	158.4	23999	11000	6945
TX76	CAR - A2000X - rep 2	156.9	23999	9900	6311
TX77	CAR - A2000X - rep 3	154.6	23999	9800	6340
TX104	DAW - A0.2X - rep 1	146.9	2.3	2	1.36
TX105	DAW - A0.2X - rep 2	147.5	2.3	2.3	1.56
TX106	DAW - A0.2X - rep 3	150.2	2.3	1.9	1.26
TX107	DAW - A0.4X	148.2	4.7	2.7	1.82
TX108†	DAW - A0.8X	145.9	9.3	6.1	4.18
TX109	DAW - A1X	149.6	12	5.3	3.54
TX110	DAW - A10X - rep 1	150.7	117	29	19.2
TX111	DAW - A10X - rep 2	149.3	117	30	20.1
TX112	DAW - A10X - rep 3	150.8	117	25	16.6
TX129	DAW - A50X	152.5	584	160	105

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg d.w.$^{-1}$)
TX113	DAW - A150X - rep 1	153.7	1752	650	423
TX114	DAW - A150X - rep 2	149.8	1752	660	441
TX115	DAW - A150X - rep 3	152.2	1752	530	348
TX116	DAW - A2000X	153.3	23363	9900	6457
TX78	HJ1 - A0.2X	92.2	1.5	2.5	2.71
TX79	HJ1 - A0.4X - rep 1	67.6	2.9	2.9	4.29
TX80	HJ1- A0.4X - rep 2	76.3	2.9	2.8	3.67
TX81	HJ1 - A0.4X - rep 3	76.6	2.9	3.4	4.44
TX82	HJ1 - A0.8X	77.6	5.8	4.1	5.28
TX83†	HJ1 - A1X - rep 1	74.9	7	3.3	4.41
TX84	HJ1 - A1X - rep 2	74.1	7	4.1	5.54
TX85	HJ1 - A1X - rep 3	73.2	7	4.1	5.60
TX86	HJ1 - A10X	78.4	73	190	242
TX87	HJ1- A50X	80.6	365	230	285
TX88	HJ1 - A150X - rep 1	137.3	1095	770	561
TX89	HJ1 - A150X - rep 2	133.2	1095	560	420
TX90	HJ1 - A150X - rep 3	139.7	1095	730	523
TX91	HJ1 - A2000X	136.2	14599	14000	10278
TX92	HJ2 - A0.2X	73.9	1.1	2	2.71
TX93†	HJ2 - A0.4X	74.9	2.2	1.9	2.54
TX94	HJ2- A0.8X - rep 1	75.0	4.4	4.4	5.86
TX95†	HJ2- A0.8X - rep 2	73.9	4.4	5.8	7.84
TX96	HJ2- A0.8X - rep 3	73.6	4.4	4	5.43
TX97	HJ2 - A1X	74.3	5.4	5.6	7.54
TX98	HJ2 - A10X	76.8	54	28	36.5
TX99	HJ2- A50X - rep 1	76.6	272	180	235
TX100	HJ2- A50X - rep 2	76.8	272	190	247

Unique ID	Sample ID	Gravimetric Moisture (%)	Nominal Concentration (mg a.i. kg^{-1})	Measured Concentration (mg a.i. kg^{-1})	Moisture Corrected Concentration (mg a.i. kg d.w.$^{-1}$)
TX101	HJ2- A50X - rep 3	78.6	272	190	242
TX102	HJ2 - A150X	76.2	816	550	722
TX103	HJ2 - A2000X	76.0	10885	8800	11575
TX117	LS - A0.2X	128.0	1.3	1.9	1.48
TX118	LS - A0.4X - rep 1	123.2	2.6	3.3	2.68
TX119	LS - A0.4X - rep 2	128.1	2.6	2.4	1.87
TX120	LS - A0.4X - rep 3	126.3	2.6	3.8	3.01
TX121	LS - A0.8X	132.5	5.2	5.5	4.15
TX122	LS - A1X	126.3	7	6.3	4.99
TX123	LS - A10X	137.3	66	110	80.1
TX124	LS - A50X	150.7	328	160	106
TX125	LS - A150X	146.6	984	430	293
TX126	LS - A2000X - rep 1	134.8	13120	7100	5267
TX127	LS - A2000X - rep 2	134.6	13120	7800	5758
TX128	LS - A2000X - rep 3	133.9	13120	5600	4184

† Measured concentration omitted because not consistent with adjacent concentration values

APPENDIX C: MODEL PARAMETERS AND EFFECTIVE DOSE CONCENTRATIONS FOR 28-DAY SURVIVAL AND REPRODUCTION TOXICITY TESTS

List of Tables

Table C.1. Dose-response curve model parameters for the 28-day survival and reproduction toxicity tests determining the toxicity of triclopyr in five soils representative of Yukon Territory ROWs to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Missing endpoints are due to an inability to model the data. SE refers to standard error of the mean.

Soil	Species	Endpoint	Model Parameters								Residual Std Error	p-value	n
			b	± SE	c	± SE	d	± SE	e	± SE			
ART	*F. candida*	Survival	6.94	± 8.52	-	-	0.96	± 0.009	544	± 307	0.05	0.0001	49
ART	*F. candida*	Reproduction	0.71	± 0.16	-	-	395	± 25.2	207	± 39.5	52.6	0.02	49
CAR	*E. crypticus*	Survival	3.87	± 2.77	-	-	0.90	± 0.05	1826	± 338	0.17	0.76	29
CAR	*E. crypticus*	Reproduction	0.59	± 0.15	-	-	1742	± 131	837	± 249	304	0.12	29
CAR	*F. candida*	Survival	6.96	± 1.65	-	-	0.96	± 0.008	3333	± 147	0.05	0.03	49
CAR	*F. candida*	Reproduction	0.94	± 0.65	-	-	29.9	± 4.32	1979	± 990	18.2	0.49	49
DAW	*E. crypticus*	Survival	5.42	± 2.04	-	-	0.91	± 0.01	277	± 56.2	0.05	0.01	39
DAW	*E. crypticus*	Reproduction	2.34	± 0.84	-	-	807	± 24.7	199	± 31.1	90.7	0.85	39
DAW	*F. candida*	Survival	6.83	± 1.60	-	-	0.94	± 0.02	1308	± 59.0	0.11	0.04	50
DAW	*F. candida*	Reproduction	2.21	± 0.27	-	-	1105	± 18.0	281	± 15.3	86.4	0.94	50
HJ1	*E. crypticus*	Survival	9.49	± 7.45	-	-	0.82	± 0.03	1607	± 91.2	0.15	0.06	45
HJ1	*E. crypticus*	Reproduction	6.31	± 2.13	-	-	1118	± 32.4	1382	± 109	177	0.001	45
HJ1	*F. candida*	Survival	1.69	± 0.44	-	-	0.97	± 0.04	3155	± 356	0.21	3.89E-14	50
HJ1	*F. candida*	Reproduction	1.26	± 0.23	-	-	662	± 21.0	1115	± 107	85.3	0.11	50
HJ2	*E. crypticus*	Survival	6.17	± 1.45	-	-	0.98	± 0.003	3497	± 260	0.07	0.09	24
HJ2	*E. crypticus*	Reproduction	5.02	± 1.33	-	-	1254	± 36.6	344	± 285	133	0.18	24
HJ2	*F. candida*	Survival†	-7.31	± 3.30	-	-	0.90	± 0.02	3357	± 462	0.13	0.18	47
HJ2	*F. candida*	Reproduction	0.99	± 0.31	-	-	392	± 27.2	1567	± 357	97.9	0.24	47
HJ2	*O. nitens*	Survival	10.0	± 2.54	-	-	0.92	± 0.02	2345	± 41.6	0.13	0.89	50
HJ2	*O. nitens*	Reproduction	3.97	± 2.12	-	-	88.2	± 4.66	1965	± 118	25.5	0.21	50
LS	*E. crypticus*	Survival	5.39	± 5.23	-	-	0.92	± 0.02	360	± 24.8	0.06	0.11	22
LS	*E. crypticus*	Reproduction ‡	13.9	± 49.0	-	-	1100	± 65.9	338	± 14.1	208	0.01	22
LS	*F. candida*	Survival	7.44	± 2.37	-	-	0.94	± 0.01	899	± 70.1	0.08	0.11	49
LS	*F. candida*	Reproduction	1.10	± 0.13	-	-	809	± 23.3	261	± 24.4	93.5	0.001	49

† Three parameter log-normal distribution; ‡ Three parameter log-logistic distribution; § Four parameter Weibull distribution

Table C.2. Effective dose concentration estimates for the 28-day survival and reproduction toxicity tests determining the toxicity of triclopyr in five soils representative of Yukon Territory ROWs to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Missing endpoints are due to inability to model data. SE refers to standard error of the mean.

Soil	Species	Endpoint	Effective Dose Concentrations (mg a.i. kg d.w.$^{-1}$)							
			ED5	± SE	ED10	± SE	ED25	± SE	ED50	± SE
ART	*F. candida*	Survival	354	± 33.1	393	± 72.0	454	± 159	516	± 259
ART	*F. candida*	Reproduction	3.13	± 3.31	8.65	± 3.30	35.7	± 18.1	124	± 31.8
CAR	*E. crypticus*	Survival	847	± 341	1021	± 280	1323	± 156	1661	± 218
CAR	*E. crypticus*	Reproduction	5.35	± 7.91	18	± 21.3	100	± 75.0	449	± 182
CAR	*F. candida*	Survival	2175	± 121	2412	± 80.8	2786	± 54.7	3162	± 129
CAR	*F. candida*	Reproduction	82.8	± 185	179	± 308	523	± 541	1338	± 750
DAW	*E. crypticus*	Survival	160	± 42.9	183	± 43.5	220	± 46.0	259	± 52.0
DAW	*E. crypticus*	Reproduction	55.9	± 18.9	76.0	± 17.8	117	± 12.7	170	± 20.0
DAW	*F. candida*	Survival	847	± 84.8	941	± 74.0	1090	± 58.3	1240	± 54.0
DAW	*F. candida*	Reproduction	73.3	± 13.4	102	± 14.7	160	± 15.4	238	± 14.8
HJ1	*E. crypticus*	Survival	1172	± 351	1265	± 303	1408	± 219	1545	± 131
HJ1	*E. crypticus*	Reproduction	863	± 106	967	± 88.0	1134	± 69.7	1304	± 88.4
HJ1	*F. candida*	Survival	546	± 215	836	± 240	1512	± 222	2541	± 222
HJ1	*F. candida*	Reproduction	106	± 53.0	188	± 74.3	416	± 105	834	± 114
HJ2	*E. crypticus*	Survival	2160	± 221	2428	± 202	2857	± 191	3295	± 227
HJ2	*E. crypticus*	Reproduction	1851	± 225	2136	± 197	2609	± 175	3108	± 231
HJ2	*F. candida*	Survival†	2681	± 492	2817	± 477	3061	± 459	3357	± 462
HJ2	*F. candida*	Reproduction	77.9	± 82.6	161	± 135	445	± 240	1082	± 326
HJ2	*O. nitens*	Survival	1744	± 122	1874	± 98.4	2072	± 60.8	2263	± 37.0
HJ2	*O. nitens*	Reproduction	931	± 383	1115	± 353	1436	± 267	1792	± 149
LS	*E. crypticus*	Survival	208	± 97.9	237	± 81.1	286	± 46.3	337	± 8.18
LS	*E. crypticus*	Reproduction ‡	274	± 201	289	± 158	313	± 84.0	338	± 14.1
LS	*F. candida*	Survival	603	± 98.8	664	± 91.2	760	± 79.2	856	± 71.0
LS	*F. candida*	Reproduction	17.5	± 6.52	34	± 9.97	84.2	± 16.2	187	± 21.5

† Three parameter log-normal distribution; ‡ Three parameter log-logistic distribution; § Four parameter Weibull distribution

Table C.3. Dose-response curve model parameters for the 28-day survival and reproduction toxicity tests determining the toxicity of imazapyr in five soils representative of Yukon Territory ROWs to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Missing endpoints are due to an inability to model the data. SE refers to standard error of the mean.

Soil	Species	Endpoint	Model Parameters						Residual Std Error	p-value	n
			b	± SE	d	± SE	e	± SE			
ART	*F. candida*	Survival	4.69	± 9.49	0.89	± 0.02	509	± 397	0.14	0.58	42
ART	*F. candida*	Reproduction	11.2	± 33.1	362	± 11.6	325	± 64.9	66.7	0.37	42
CAR	*E. crypticus*	Survival	5.19	± 9.32	0.95	± 0.02	553	± 472	0.06	0.06	21
CAR	*E. crypticus*	Reproduction	1.73	± 4.66	1528	± 44.1	1434	± 5571	159	0.96	21
CAR	*F. candida*	Survival	1.18	± 0.55	0.95	± 0.02	8515	± 1456	0.11	0.75	45
DAW	*E. crypticus*	Survival	0.83	± 0.24	0.92	± 0.04	3710	± 1036	0.16	0.97	33
DAW	*E. crypticus*	Reproduction	0.57	± 0.13	1114	± 52.5	1166	± 331	154	0.15	33
HJ1	*E. crypticus*	Survival	0.72	± 0.31	0.93	± 0.06	8611	± 2984	0.19	0.98	26
HJ1	*E. crypticus*	Reproduction	0.80	± 0.17	1194	± 51.4	2945	± 946	176	0.19	26
HJ1	*F. candida*	Survival	2.54	± 1.27	0.98	± 0.005	6125	± 1613	0.03	0.61	44
HJ2	*E. crypticus*	Reproduction	0.49	± 0.23	1118	± 38.7	39773	± 38469	110	0.84	28
HJ2	*F. candida*	Reproduction	1.56	± 0.67	450	± 12.2	904	± 154	63.4	0.02	45
HJ2	*O. nitens*	Survival	3.34	± 4.52	0.84	± 0.03	7587	± 5043	0.17	0.004	44
LS	*F. candida*	Reproduction	0.83	± 0.21	674	± 25.4	2365	± 757	119	0.0009	42

Table C.4. Effective dose concentration estimates for the 28-day survival and reproduction toxicity tests determining the toxicity of imazapyr in five soils representative of Yukon Territory ROWs to *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. Missing endpoints are due to inability to model data. SE refers to standard error of the mean.

Soil	Species	Endpoint	Effective Dose Concentrations (mg a.i. kg d.w.$^{-1}$)							
			ED5	± SE	ED10	± SE	ED25	± SE	ED50	± SE
ART	*F. candida*	Survival	270	± 140	315	± 71.3	315	± 71.3	471	± 294
ART	*F. candida*	Reproduction	249	± 243	266	± 209	291	± 153	314	± 92.8
CAR	*E. crypticus*	Survival	312	± 69.6	359	± 48.8	435	± 188	516	± 375
CAR	*E. crypticus*	Reproduction	259	± 247	392	± 264	699	± 1395	1161	± 3859
CAR	*F. candida*	Survival	691	± 726	1270	± 981	2969	± 1157	6245	± 791
DAW	*E. crypticus*	Survival	105	± 121	249	± 226	832	± 483	2390	± 809
DAW	*E. crypticus*	Reproduction	6.68	± 7.93	23.3	± 21.3	132	± 73.7	617	± 191
HJ1	*E. crypticus*	Survival	140	± 253	381	± 529	1531	± 1270	5181	± 2169
HJ1	*E. crypticus*	Reproduction	71.3	± 59.8	176	± 116	618	± 274	1861	± 608
HJ1	*F. candida*	Survival	1900	± 1610	2523	± 1779	3748	± 1901	5301	± 1774
HJ2	*E. crypticus*	Reproduction	88.9	± 179	390	± 523	3074	± 1483	>11532	-
HJ2	*F. candida*	Reproduction	134	± 93.9	213	± 108	407	± 100	715	± 84.1
HJ2	*O. nitens*	Survival	3120	± 5543	3870	± 5783	5226	± 5793	6799	± 5356
LS	*F. candida*	Reproduction	65.3	± 67.4	156	± 129	525	± 291	1519	± 555

APPENDIX D: TRICLOPYR AND IMAZAPYR DOSE RESPONSE CURVES FOR 28-DAY INVERTEBRATE TOXICITY TESTS (SURVIVAL AND REPRODUCTION ENDPOINTS)

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Figure D.23. Response of *F. candida* survival and reproduction to imazapyr concentrations in LS soil in 28-day toxicity assay.

Figure D.24. Response of *F. candida* survival and reproduction to imazapyr concentrations in Artificial soil in 28-day toxicity assay.

Figure D.25. Response of *O. nitens* survival and reproduction to imazapyr concentrations in HJ2 soil in 28-day toxicity assay.

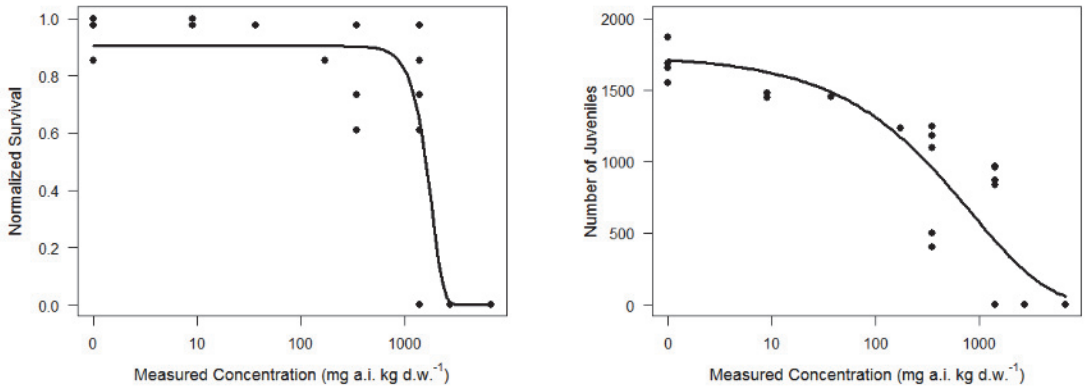

Figure D.1. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in CAR soil in 28-day toxicity assay.

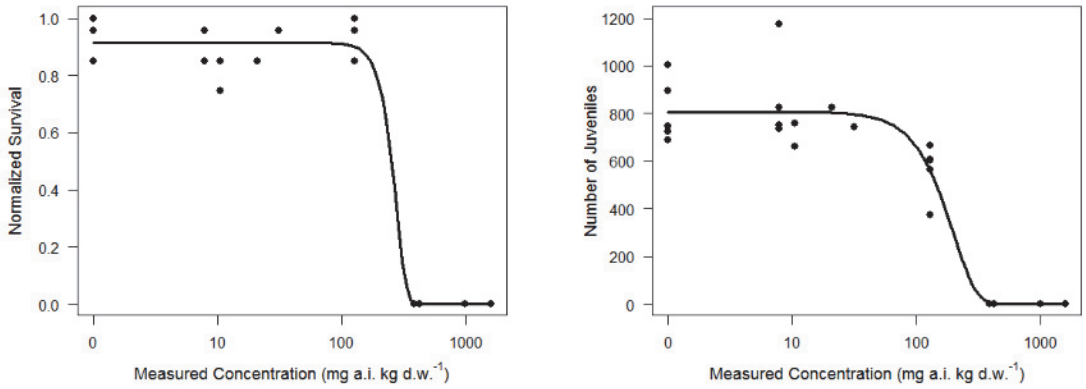

Figure D.2. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in DAW soil in 28-day toxicity assay.

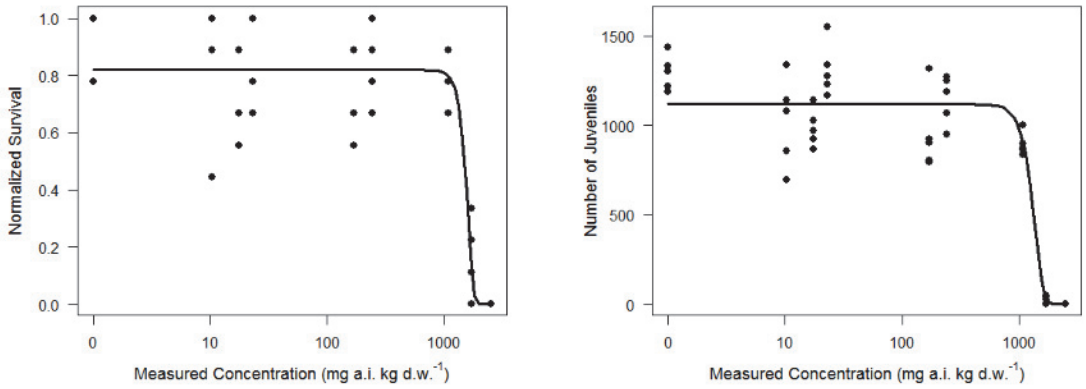

Figure D.3. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in HJ1 soil in 28-day toxicity assay.

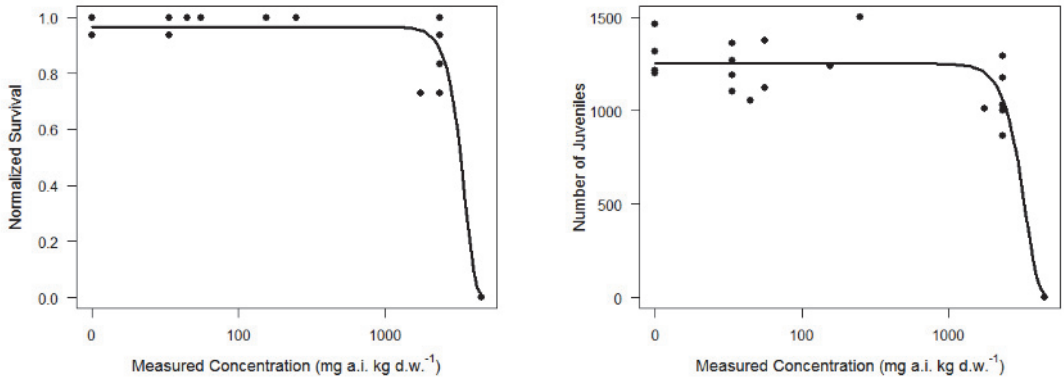

Figure D.4. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in HJ2 soil in 28-day toxicity assay.

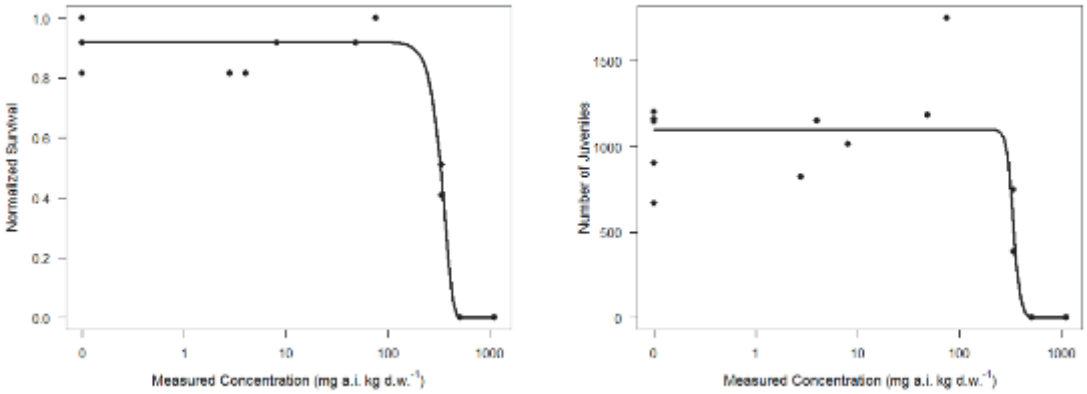

Figure D.5. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in LS soil in 28-day toxicity assay.

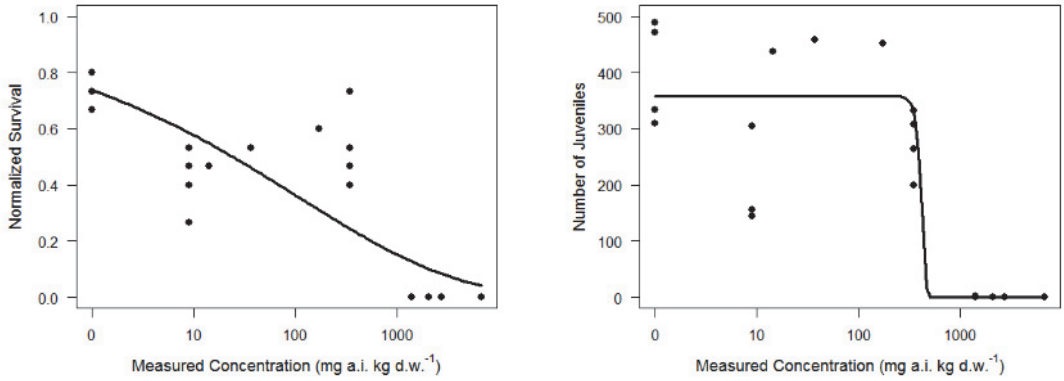

Figure D.6. Response of *E. crypticus* survival and reproduction to triclopyr concentrations in Artificial soil in 28-day toxicity assay.

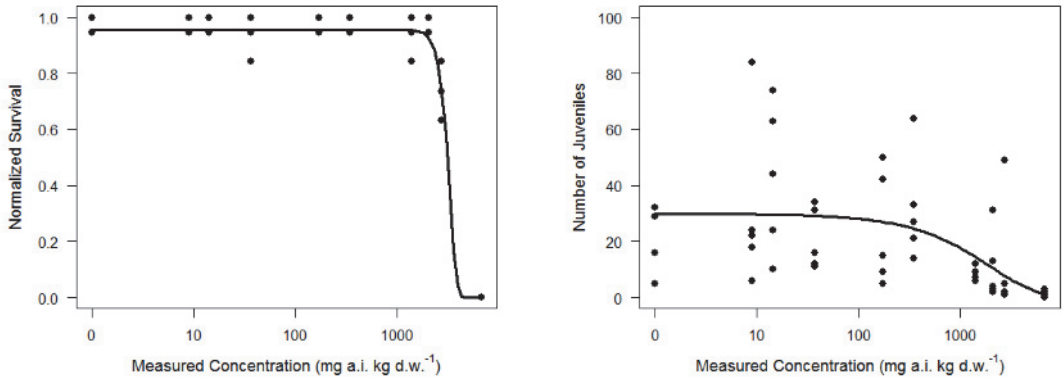

Figure D.7. Response of *F. candida* survival and reproduction to triclopyr concentrations in CAR soil in 28-day toxicity assay.

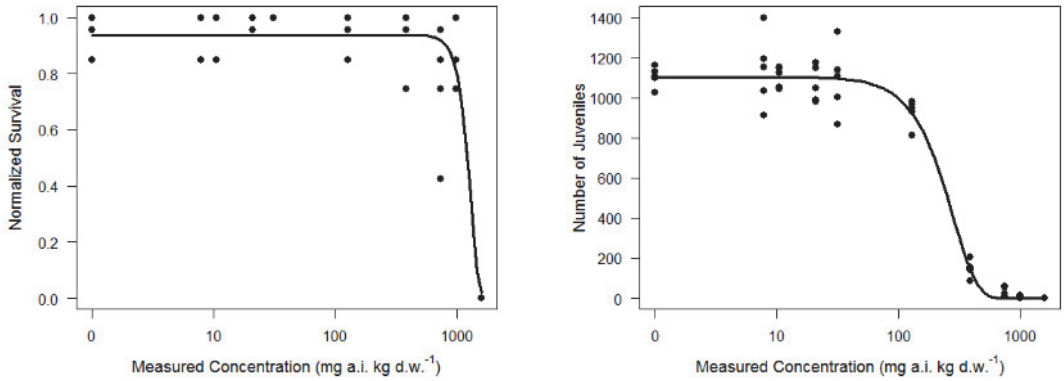

Figure D.8. Response of *F. candida* survival and reproduction to triclopyr concentrations in DAW soil in 28-day toxicity assay.

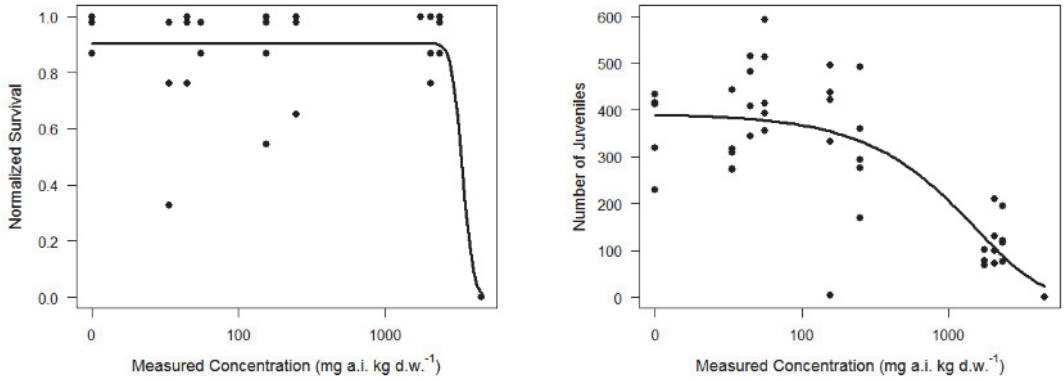

Figure D.9. Response of *F. candida* survival and reproduction to triclopyr concentrations in HJ1 soil in 28-day toxicity assay.

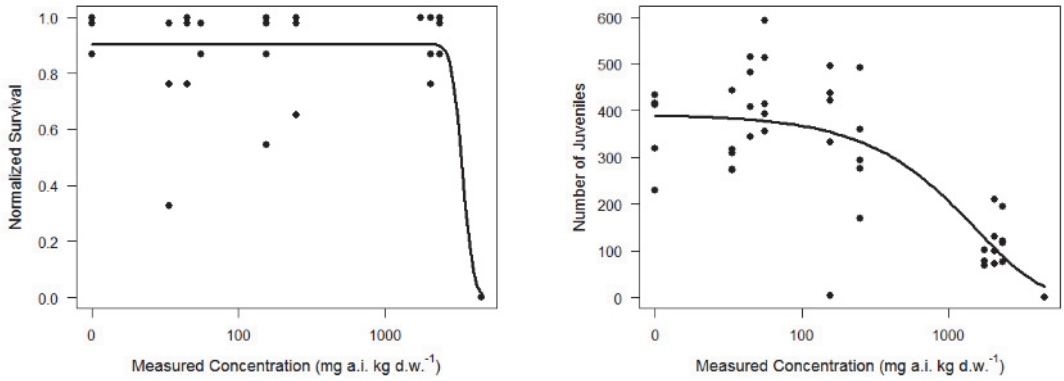

Figure D.10. Response of *F. candida* survival and reproduction to triclopyr concentrations in HJ2 soil in 28-day toxicity assay.

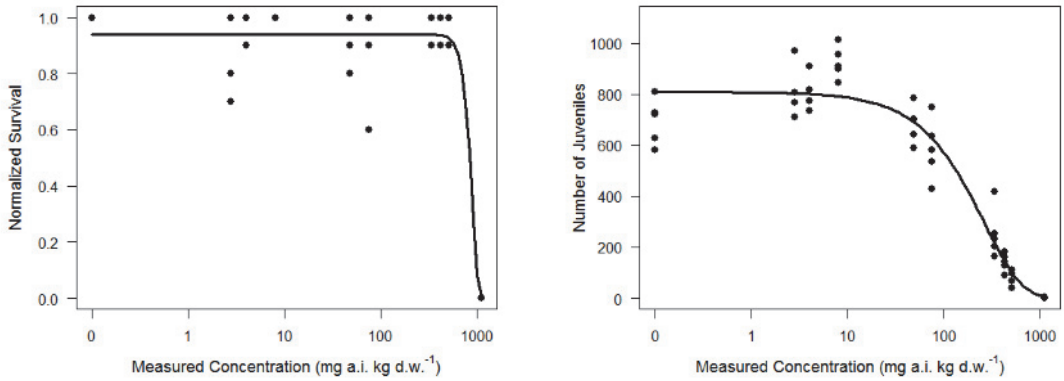

Figure D.11. Response of *F. candida* survival and reproduction to triclopyr concentrations in LS soil in 28-day toxicity assay.

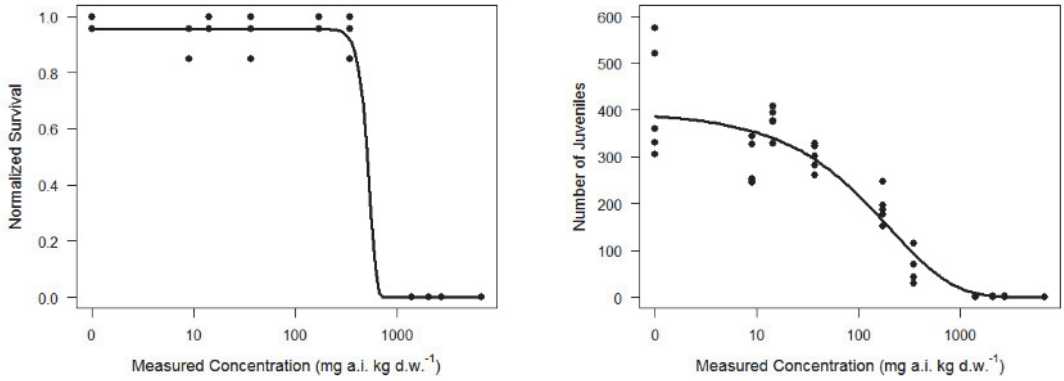

Figure D.12. Response of *F. candida* survival and reproduction to triclopyr concentrations in Artificial soil in 28-day toxicity assay.

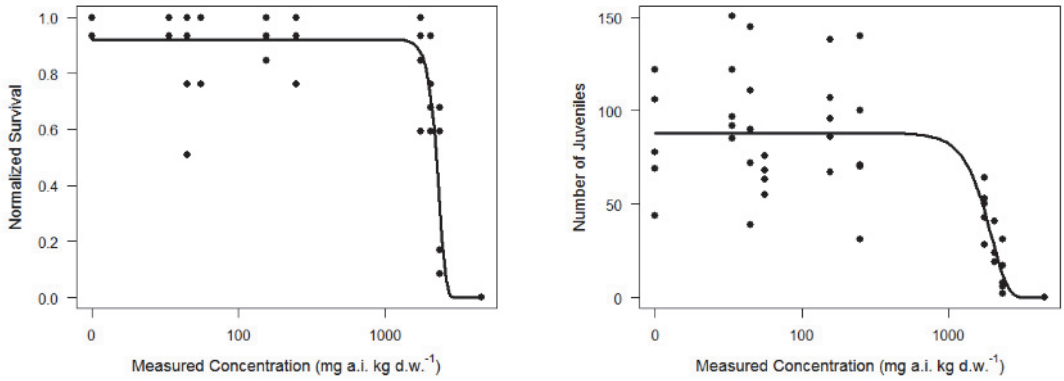

Figure D.13. Response of *O. nitens* survival and reproduction to triclopyr concentrations in HJ2 soil in 28-day toxicity assay.

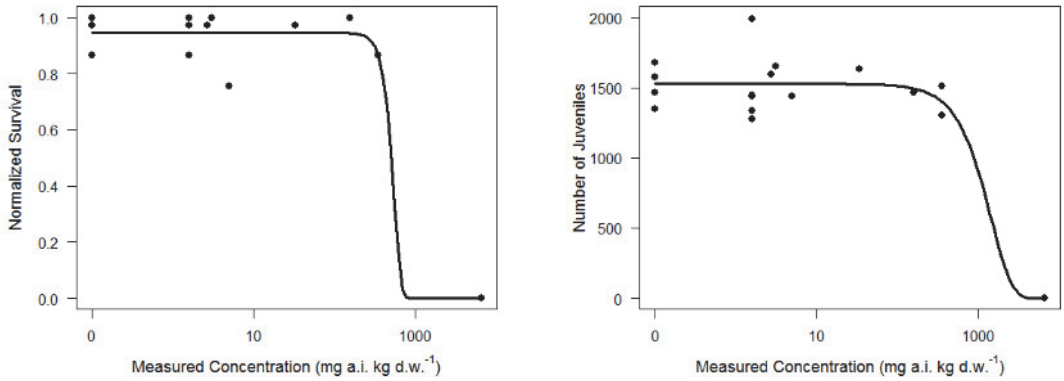

Figure D.14. Response of *E.crypticus* survival and reproduction to imazapyr concentrations in CAR soil in 28-day toxicity assay.

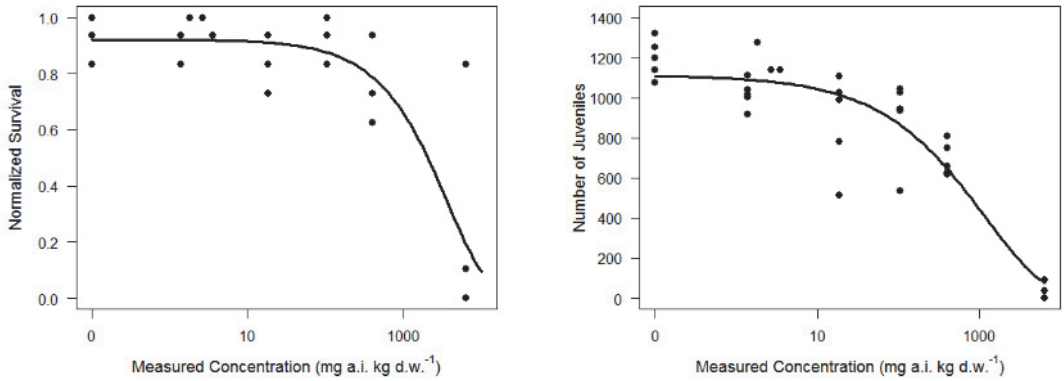

Figure D.15. Response of *E.crypticus* survival and reproduction to imazapyr concentrations in DAW soil in 28-day toxicity assay.

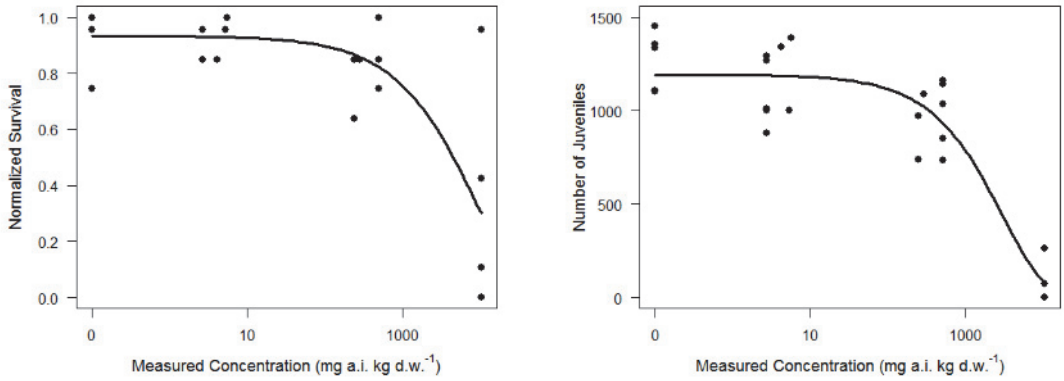

Figure D.16. Response of *E.crypticus* survival and reproduction to imazapyr concentrations in HJ1 soil in 28-day toxicity assay.

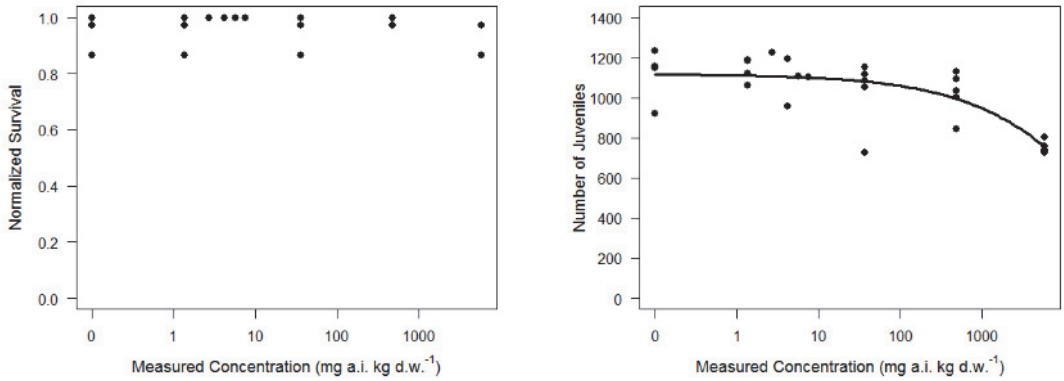

Figure D.17. Response of *E.crypticus* survival and reproduction to imazapyr concentrations in HJ2 soil in 28-day toxicity assay.

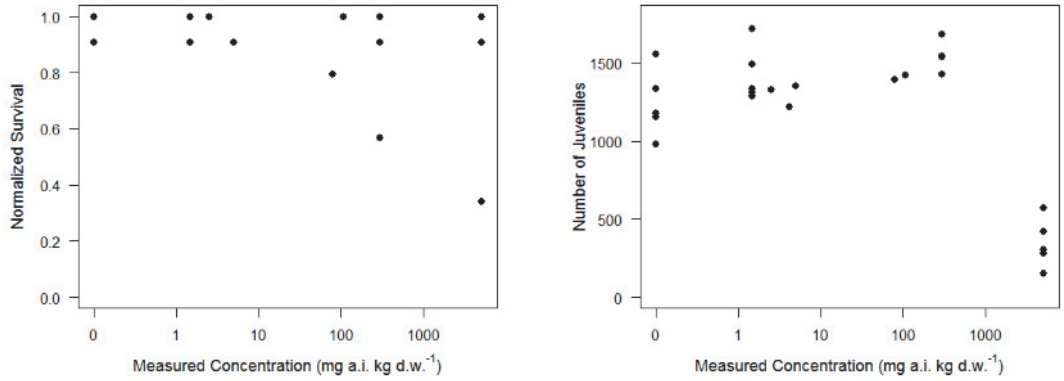

Figure D.18. Response of *E.crypticus* survival and reproduction to imazapyr concentrations in LS soil in 28-day toxicity assay.

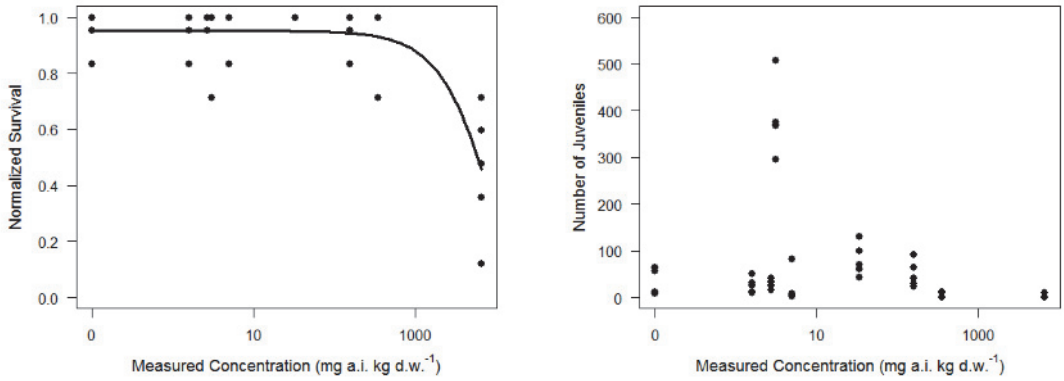

Figure D.19. Response of *F. candida* survival and reproduction to imazapyr concentrations in CAR soil in 28-day toxicity assay.

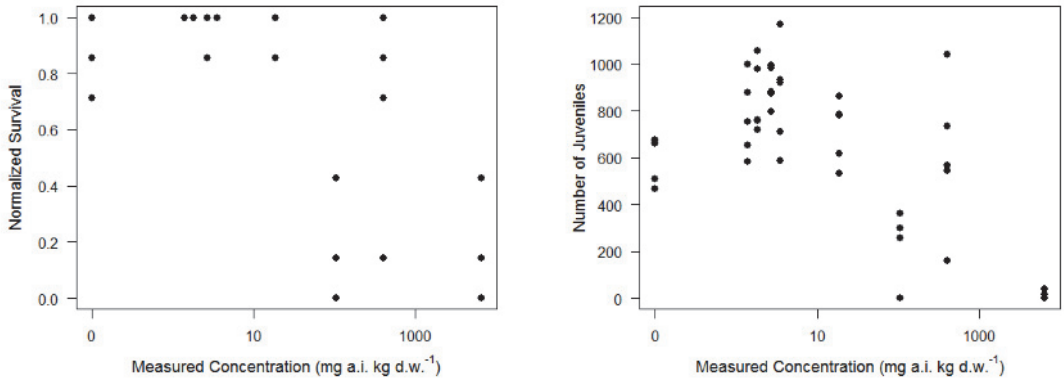

Figure D.20. Response of *F. candida* survival and reproduction to imazapyr concentrations in DAW soil in 28-day toxicity assay.

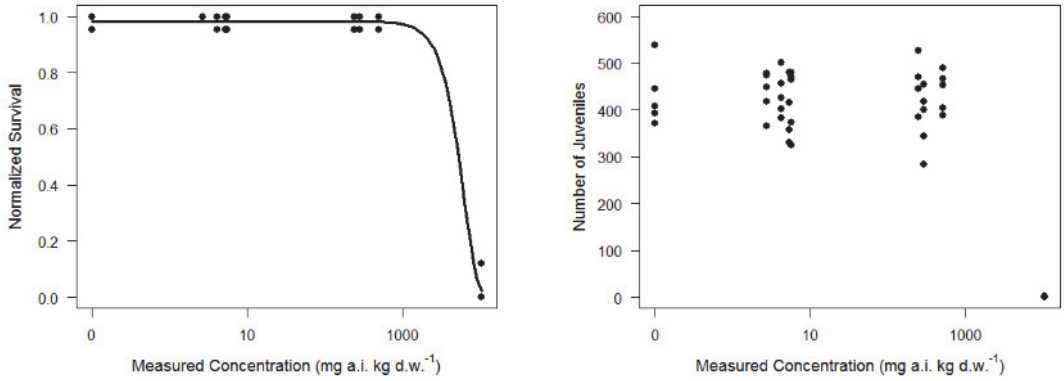

Figure D.21. Response of *F. candida* survival and reproduction to imazapyr concentrations in HJ1 soil in 28-day toxicity assay.

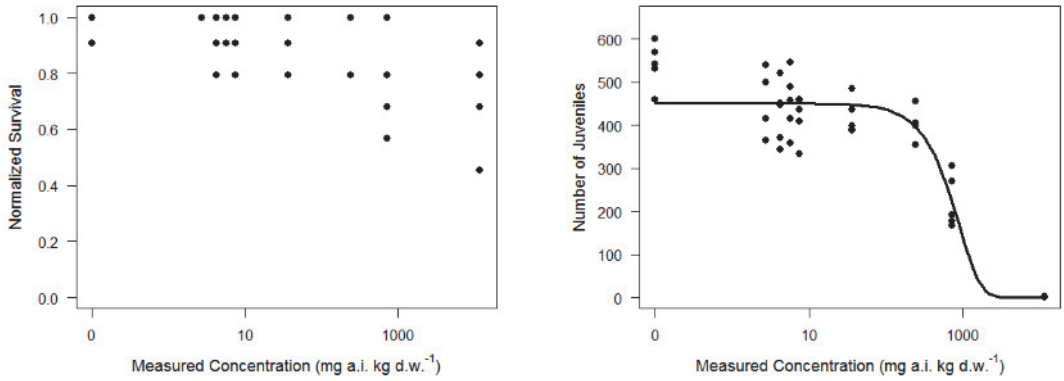

Figure D.22. Response of *F. candida* survival and reproduction to imazapyr concentrations in HJ2 soil in 28-day toxicity assay.

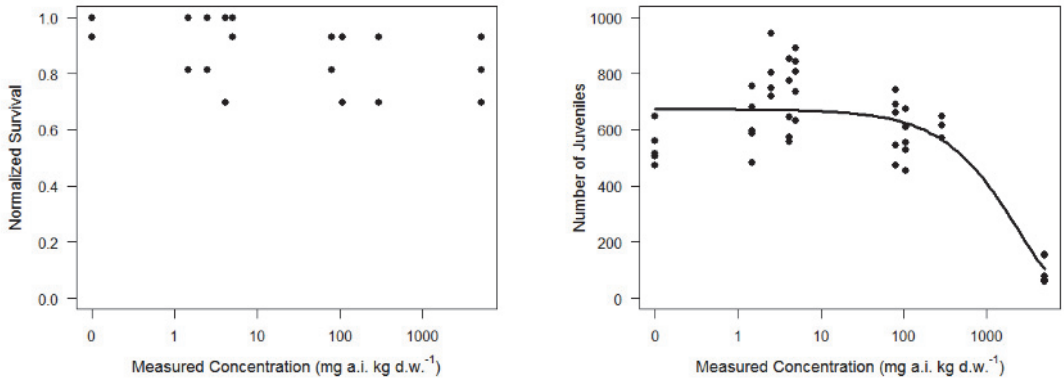

Figure D.23. Response of *F. candida* survival and reproduction to imazapyr concentrations in LS soil in 28-day toxicity assay.

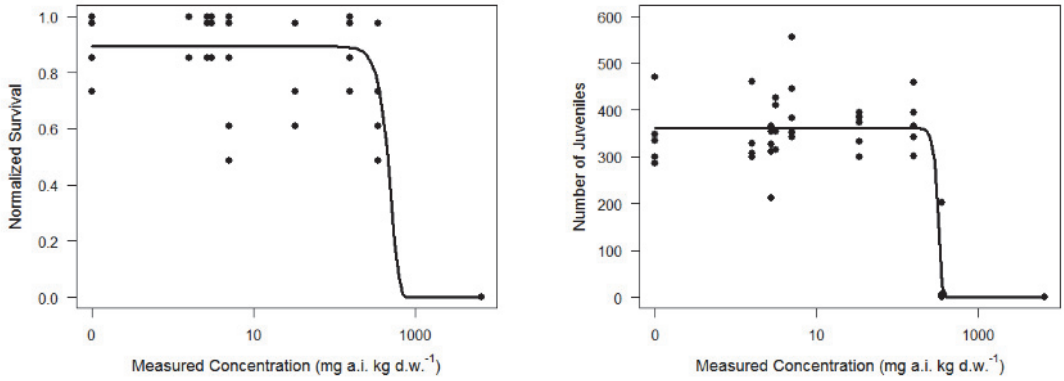

Figure D.24. Response of *F. candida* survival and reproduction to imazapyr concentrations in Artificial soil in 28-day toxicity assay.

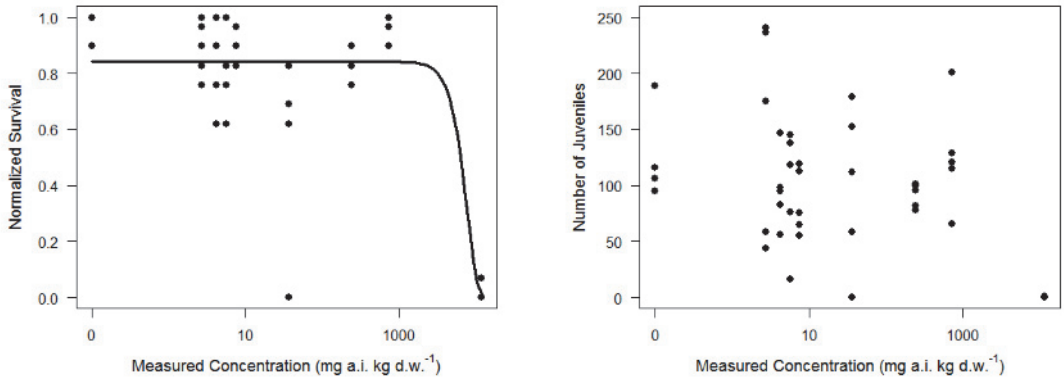

Figure D.25. Response of *O. nitens* survival and reproduction to imazapyr concentrations in HJ2 soil in 28-day toxicity assay.

APPENDIX E: DOSE-RESPONSE DATA FOR PHOSPHATASE, ARYLSULFATASE AND B-GLUCOSIDASE ACTIVITY AFTER EXPOSURE TO TRICLOPYR AND IMAZAPYR.

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Site	Calculated Field Application Rate	Measured Concentration (mg a.i. kg d.w.$^{-1}$) for Each Dose Level									
	(mg a.i. kg^{-1})	1	2	3	4	5	6	7	8	9	10†
CAR‡	75.5	0.00	14.3¶	36.8	173¶	347	1399¶	2064¶	2728	6725	13449§
DAW‡	73.5	0.00	7.90	10.5	21.0	129	387	691	995	1592	6366
HJ1	45.9	0.00	10.3	17.5	23.0	244	1090	1401	1712	2502	10006
HJ2	34.2	0.00	33.6	44.6	55.6	249	1753	2050	2347	4518	18073
LS	41.3	0.00	2.8	4.00	8.10	75.5	337	426§	514	1114	4459

Table E.2. Measured concentrations (mg a.i. kg d.w^{-1}) of imazapyr for each dose level used for the arylsulfatase, B-glucosidase and phosphatase enzyme assays. Field application rate was calculated using the maximum field application rate (720 g imazapyr ha^{-1}) and the bulk densities (g/cm^3) for each site. Dose levels (#1-9) used were based on the results of the range finding tests. Dose level 1 represents the negative control. (†) B-glucosidase was not completed using HJ1 soil. (‡) Arylsulfatase and phosphatase assays were not competed used LS soil. (§) represents concentrations that were not measured and were estimated from adjacent concentrations.

Site	Calculated Field Application Rate	Measured Concentration (mg a.i. kg d.w.$^{-1}$) for Each Dose Level								
	(mg a.i. kg^{-1})	1	2	3	4	5	6	7	8	9
HJ1†	7.3	0.00	2.71	4.13	5.28	5.57	242	285	501	10278
HJ2	5.44	0.00	2.71	4.18§	5.65	7.54	36.5	241	722	11515
LS‡	6.56	0.00	1.48	2.52	4.15	4.99	80.1	106	293	5069

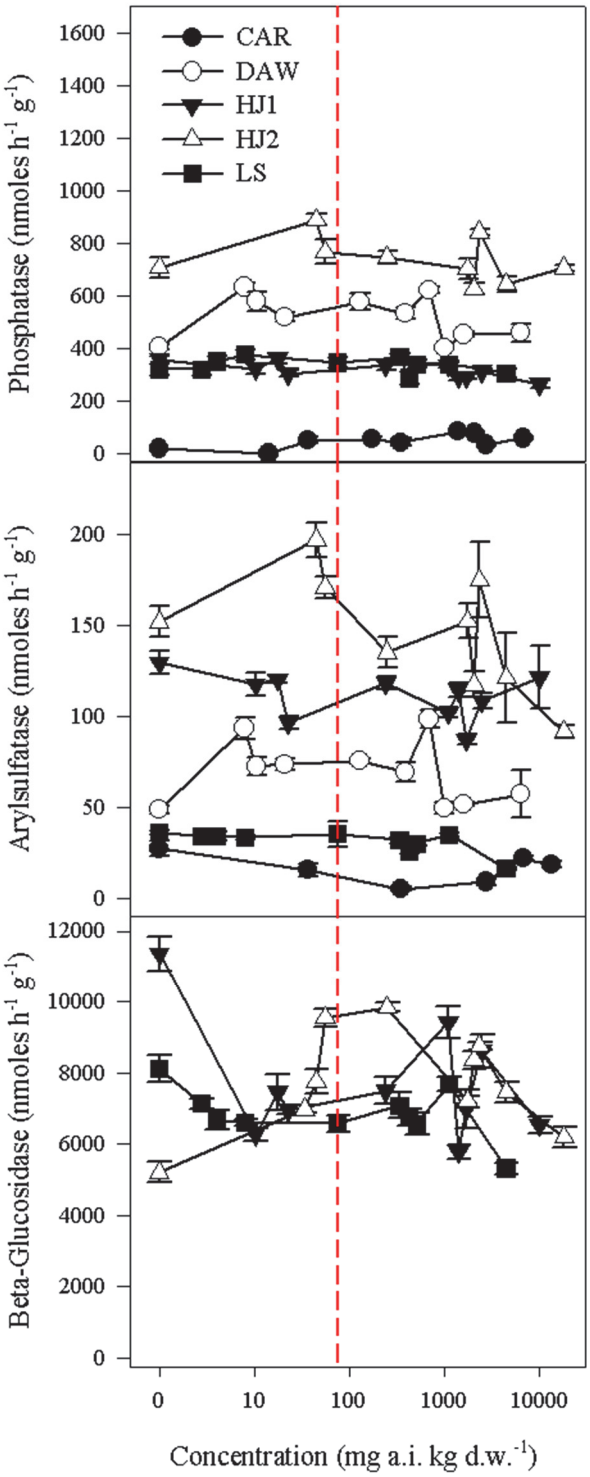

Figure E.1. Phosphatase, arylsulfatase and B-glucosidase activity in response to increasing concentrations of triclopyr in select Yukon Territory soils. Symbols represent the mean (n=5) while the error bars represent the standard error of the mean. Red dashed line represents the calculated maximum application rate (75.5 mg triclopyr kg d.w.$^{-1}$).

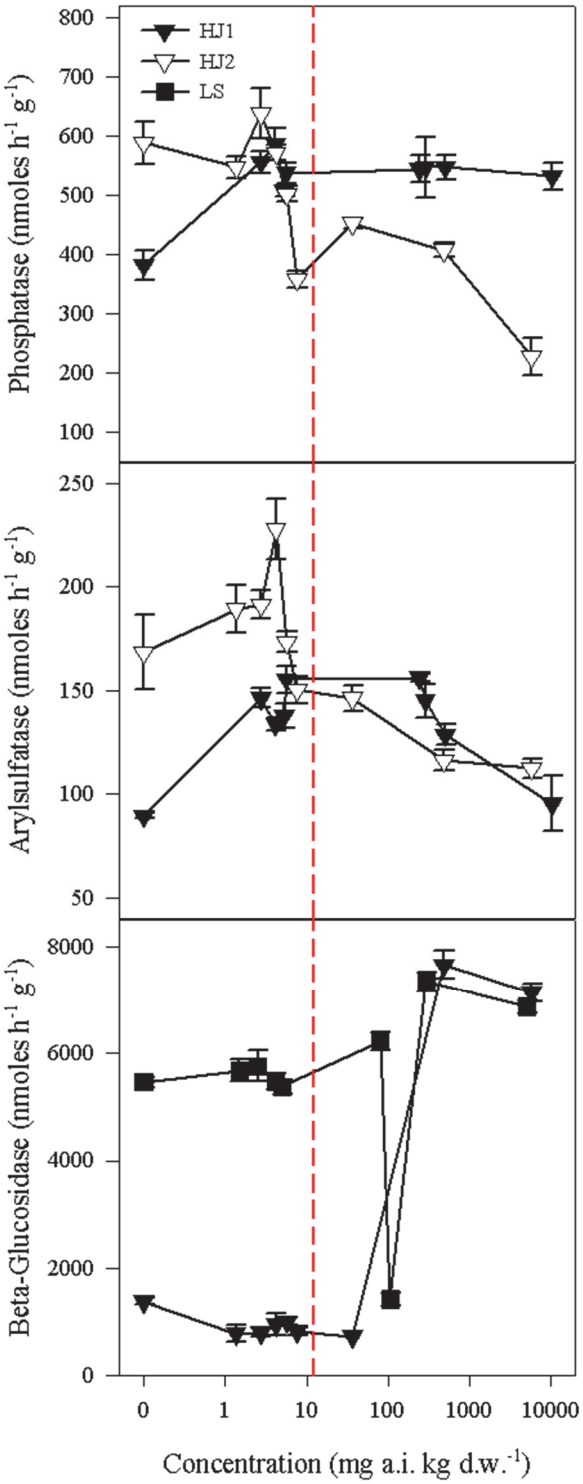

Figure E.2. Phosphatase, arylsulfatase and B-glucosidase activity in response to increasing concentrations of imazapyr in select Yukon Territory soils. Symbols represent the mean (n=5) while the error bars represent the standard error of the mean. Red dashed line represents the calculated maximum application rate (12 mg imazapyr kg d.w.$^{-1}$).

APPENDIX F: EFFECTS OF IMAZAPYR TO *FOLSOMIA CANDIDA* SURVIVAL AND REPRODUCTION OVER TIME

INTRODUCTION

Imazapyr is a non-selective herbicide that elicits toxic responses in plants by inhibiting acetolactate synthase (ALS), an enzyme only found in plants (Stidham, 1991; Masson and Webster, 2001; Heiser, 2007; Senseman, 2007). Imazapyr is known to be effective at controlling woody deciduous trees, such as *Populus spp*. and *Salix spp.* (Wang et al., 2005; Ramezani et al., 2010; EDI, 2013; Gianelli et al., 2014). If employed along right-of ways (ROWs) in the Yukon Territory, imazapyr has the potential to increase long-term control of target vegetation resulting in reduced management costs (EDI, 2013). However, imazapyr is known to have long residence times in soil (Senseman, 2007; Douglass et al., 2016b) with residues detected up to 456 days after treatment in northern climates (Newton et al., 2008). The slow dissipation of imazapyr has pronounced effects on non-target vegetation species (Douglass et al., 2016b; Isbister, 2016). Non-target vegetation continued to be effected two years after the application of imazapyr when applied at the maximum field application rate (Isbister, 2016).

While highly potent to vegetation, little is known about the toxicity of imazapyr to soil invertebrates. The results presented in this thesis indicate that imazapyr may not be toxic to the invertebrates tested including *Enchytreaus crypticus*, *Folsomia candida* and *Oppia nitens*. However, since imazapyr continues to elicit a toxic response in non-target vegetation long after application (Douglass et al., 2016a; b; Isbister, 2016) it is possible that imazapyr toxicity increases to soil invertebrates over time. It is suspected that toxicity could increase due to the presence of toxic metabolites or the presence of a potent enantiomer. There four main metabolites of imazapyr include 2,3-pyridine-dicarboxamide, 2,3-pyridine-carboxylic anhydride, 2,3-pyridine-dicarboximide and 2-(4-hydroxy-5-oxo-2-imdaolin-2-yl) nicotinic acid (Wang et al., 2006). No toxicity studies examining the metabolites alone could be found. Additionally, imazapyr is a chiral herbicide and it is well known that different enantiomers can have different toxic mechanisms (Garrison, 2006; Ramezani et al., 2010). While it hasn't been explored in detail, it is possible that one of the enantiomers could be increase the toxicity of imazapyr to soil invertebrates. For imidazolinone herbicides, it is known that the R(+) enantiomer is more herbicidally active and is preferred by microorganisms resulting in rapid dissipation (Ramezani et al., 2010). The S(-) enantiomer degrades slower and is less herbicidally active. The effects of the S(-) enantiomer on

non-target organisms has not been explored, as is the case with many less biologically active enantiomers, but it is possible that this enantiomer could elicit a toxic response (Garrison, 2006). The objective of this preliminary study was to determine if imazapyr toxicity to *F. candida* increases with soil residence time.

METHODS

Soil Sampling

Based on differences in soil properties, two sites, CAR and LS, were selected to assess the toxicity of imazapyr to *F. candida* over time. Untreated soils were collected from three to five locations at both sites. Each collection site was cleared of vegetation and coarse woody debris prior to sampling. Approximately 20 kilograms (kg) of organic soil, typically consisting of the top three centimetres (cm), was collected at each location. Only the organic layer was sampled for the laboratory toxicity tests as the invertebrates selected for the toxicity study prefer this layer. In addition, it is expected that the majority of the herbicide residues will remain in the upper soil horizon (Stephenson et al., 1990; Johnson et al., 2000; Newton et al., 2008; Douglass et al., 2016b). After collection, soils were air dried, sieved to 2 mm, homogenized, and stored at room temperature. Prior to toxicity testing the soil was pasteurized at 80^{o}C for 48 hours. Random grab samples (n=5) from the bulk soil for each site were analyzed for soil moisture, total nitrogen, total organic carbon and pH. Soil moisture was analyzed using a Mettler Toledo MJ33 Moisture Analyzer (Mettler Toledo Canada, Mississauga, ON). Total nitrogen was analyzed using the LECO TruMac CNS analyzer (LECO Corporation., St. Joseph, MI), total organic carbon was analyzed using the C-632 LECO Carbon analyzer (LECO Corporation., St. Joseph, MI) and pH was measured using a 0.01M calcium chloride extraction. Table 4.1 summarizes specific soil properties obtained from the organic soil collected at each site. Full soil characterization methodology can be found in Appendix A.

Soil Toxicity Tests

F. candida cultures were maintained in the Soil Toxicology Laboratory at the University of Saskatchewan, Saskatoon, Saskatchewan. Cultures were kept in the dark at a temperature of 20±2 ^{o}C. *F. candida* cultures were reared in a plastic culture box with a base of 5:1 plaster of Paris and activated charcoal with baker's yeast added as a food source as needed.

Arsenal® Powerline (240 g L^{-1} imazapyr isopropylamine salt; BASF Canada Inc., Mississauga, ON) was used to assess the toxicity of imazapyr over time. One concentration was selected for the tests at a level where toxic responses were expected to be observed. Doses of 33.1 mg ai kg d.w.$^{-1}$ and 80.1 mg a.i. kg d.w^{-1} were used for the CAR and LS soils, respectively. Soils were dosed with invertebrates added at the following time intervals: 0, 1, 3, 7, 14, 21, 30 and 60 days after dosing. Five replicates were included at each time interval. Intervals were selected to mimic the field dissipation study conducted at the LS site. Hasten™ Spray Adjuvant (704g L^{-1} Ethyl and Methyl esters of vegetable oil with 196 g L^{-1} non-ionic surfactants; Victorian Chemicals Group, Victoria, AUS) was used at a rate of 0.25 % by volume.

Species specific standard operating procedures were followed for the *F. candida* toxicity test (Environment Canada, 2014). Briefly, each test consisted of adding ten 10 to 12 day old individuals to a glass vessel containing a volume of 30 mL of soil wetted to 50% field water holding capacity. Test vessels were maintained at approximately 20 ± 2 ^{o}C with 12:12 hour photoperiods for 28 days. Adult survival and reproduction were assessed for each organism at 28 days.

Chemical Analysis

Soil samples were analyzed at the University of Guelph's Food and Agriculture Laboratory using High Performance Liquid Chromatography coupled with tandem Mass Spectrophotometry (HPLC-MS/MS). Soil samples were analyzed to determine measured concentrations so accurate estimations of toxicity could be established. The method detection limit and method quantification limits for imazapyr were 0.0006 mg a.i. kg^{-1} and 0.002 mg a.i. kg^{-1}, respectively, with a mean recovery rate of 75 %.

Statistical Analysis

Both the LS and CAR data sets were checked for the assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Bartlett`s test). Analysis of variance (ANOVA) methods were used to determine the difference between time intervals. The ANOVA was followed by a TukeyHSD post hoc test. Normality and homogeneity of variance were also confirmed for model residuals. Outliers were checked using the Grubbs test with zero outliers removed. Statistical analyses were completed using the R software (version 3.2.4) (R Core Team, 2016) with figures plotted using SigmaPlot 10.0.

RESULTS AND DISCUSSION

Little is known about the impact 'aged' imazapyr may have on soil invertebrate communities. However, it is hypothesized that increased toxicity could result from one of the four metabolites (2,3-pyridine-dicarboxamide, 2,3-pyridine-carboxylic anhydride, 2,3-pyridine-dicarboximide and 2-(4-hydroxy-5-oxo-2-imdaolin-2-yl) nicotinic acid) or, due to imazapyr's chiral nature, one of its enantiomers (R(+) enantiomer or S(-) enantiomer). Results from the LS soil suggest that the toxicity of imazapyr to *F. candida* may increase over time. For the CAR soil, the 0, 1, 3, 14 and 21 days after dosing tests produced less than ten juveniles which is not valid (Environment Canada, 2014). Therefore, the CAR results were not analyzed.

There was no difference in adult survival at any time interval for tests run in the LS soil (ANOVA, TukeyHSD>0.05) (Figure F.1.). Juvenile production in the LS soil had the lowest juvenile production in the 30 days after dosing replicates (ANOVA, TukeyHSD<0.05) (Figure F.2).

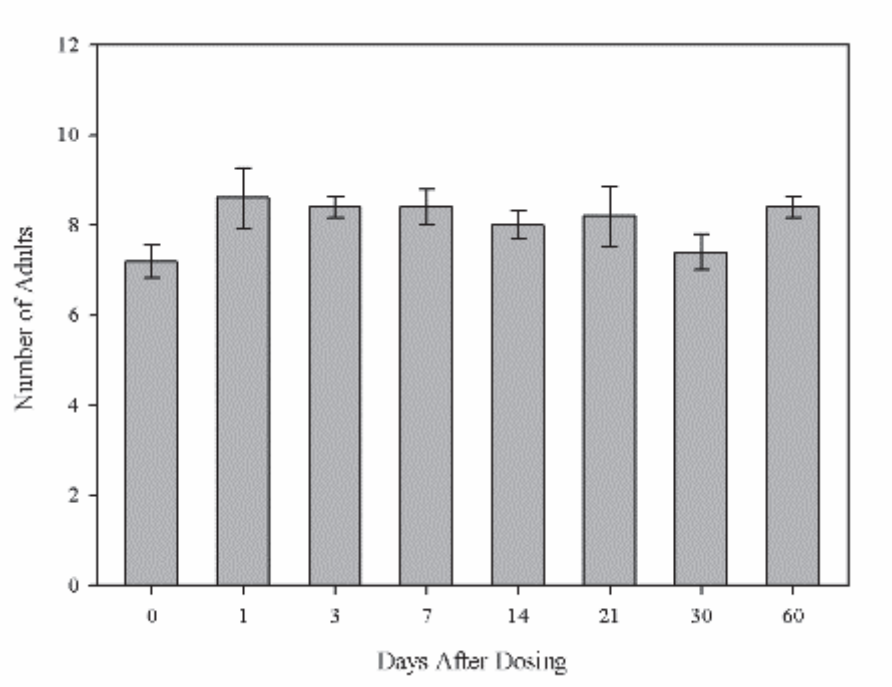

Figure F.1. *Folsomia candida* survival after exposure to imazapyr at 0, 1, 3, 7, 14, 21, 30 and 60 days after dosing in the LS site soil. Bars represent mean ± standard error (n=5) of the estimate. No significant differences (p<0.05) were observed between time intervals for each soil.

Juvenile production in the LS soil was significantly affected at some of the time intervals. Tests started 0, 1, 3 and 7 days after dosing had significantly more juveniles (p<0.05) than the 30

days after dosing. In addition, the 1 day and 60 day tests were significantly different (ANOVA<0.05) but there was no significant difference observed between 0 and 60 days after dosing. Exposure to imazapyr at 30 days after dosing had the most significant impact on *F. candida* reproduction. While not statistically significant, Figure F.2 clearly shows a decline in juvenile production in the LS soil 14 days after dosing. The number of juveniles produced in the tests was still greater than 200 indicating that, while there was a decrease, overall reproduction was not greatly impacted. More tests should be run with longer time intervals to determine if further declines in reproduction occur.

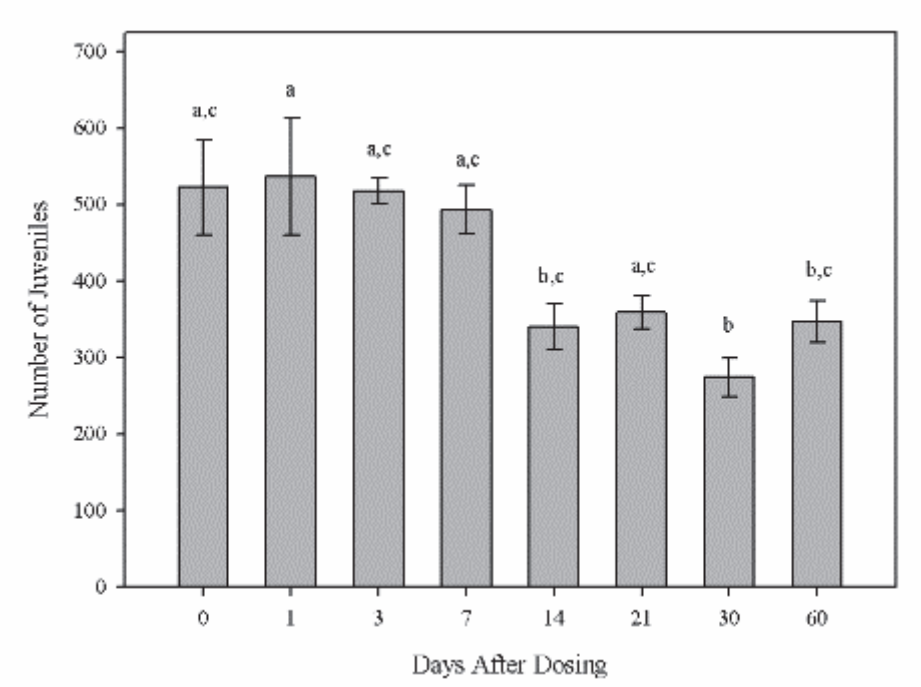

Figure F.2. *Folsomia candida* reproduction (number of juveniles produced) after exposure to imazapyr at 0, 1, 3, 7, 14, 21, 30 and 60 days after dosing in CAR and LS site soils. Bars represent mean ± standard error (n=5) of the estimate and similar letters represent no statistically significant (p>0.05) difference between intervals.

CONCLUSIONS

These findings serve as a range finding test identifying that imazapyr could become more toxic to *F. candida* after extended periods in soil. It is possible that one of the four metabolites or one of the enantiomers of imazapyr could influence the toxicity of imazapyr to soil invertebrates over time. Additional tests with longer time intervals after dosing are needed to confirm this hypothesis. Also, investigations examining the imazapyr metabolites and the enantiomers are required to determine if these compounds are eliciting a greater toxic response when soil

invertebrates are exposed to soils with 'aged' imazapyr. Further, to account for variability in the soils, two controls, one negative control and one surfactant control should have been tested simultaneously at each time interval. This would have helped to determine if the effects observed were the result of the test conditions or a sensitivity to imazapyr. The surfactant control would help determine if Hasten is contributing to the toxicity of imazapyr over time.

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APPENDIX G: TOXIC EXPOSURE RATIOS FOR CUT STUMP AND POINT INJECTION TREATMENTS.

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Table G.1. Toxic Exposure Ratios (TER) for triclopyr and imazapyr calculated using the lowest 28-day EC_{10} generated for each site soil and Potential Environmental Concentration from the soil (PEC_{soil}) at 30 and 365 Days After the Cut Stump treatment. The 28-d EC_{10} values were used to determine the TERs because all TERs calculated with 28-d EC_{25} values were above the critical trigger values. The acute TER values used PEC_{soil} values from 1 day after treatment and a critical trigger value of 10. The chronic TER values used PEC_{soil} values from 30 and 365 days after treatment and have a critical trigger value of 5. Bold and underlined font indicates TER values calculated below the critical trigger value.

Herbicide	Site	EC_{10}		30 DAT		365 DAT	
		Species	Value	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER
Triclopyr	CAR	*E. crypticus*	18 ± 21.3	0.13	41.2	ND	NC
	DAW	*E. crypticus*	76 ± 17.8	3.80	14.7	ND	NC
	HJ1	*F. candida*	188 ± 74.3	ND	NC	0.04	4273
	HJ2	*F. candida*	161 ± 135	1.10	70.8	ND	NC
Imazapyr	CAR	*E. crypticus*	392 ± 264	0.015	17267	0.007	56000
	DAW	*E. crypticus*	23.3 ± 21.3	0.002	3340	0.13	179
	HJ1	*E. crypticus*	176 ± 116	0.16	446	0.01	17600
	HJ2	*F. candida*	213 ± 108	0.28	318	0.008	26625

NC: Not calculated
ND: Non-detect

Table G.2. Toxic Exposure Ratios (TER) for triclopyr and imazapyr calculated using the lowest 28-d EC_{10} endpoint generated for each site soil and Potential Environmental Concentration from the soil (PEC_{soil}) at 30 and 365 Days After the Point Injection treatment. The 28-d EC_{10} values were used to determine the TERs because all TERs calculated with 28-d EC_{25} values were above the critical trigger values. The acute TER values used PEC_{soil} values from 1 day after treatment and a critical trigger value of 10. The chronic TER values used PEC_{soil} values from 30 and 365 days after treatment and have a critical trigger value of 5. Bold and underlined font indicates TER values calculated below the critical trigger value.

Herbicide	Site	28-d EC_{10} (mg a.i. kg d.w.$^{-1}$)		30 DAT		365 DAT	
		Species	Value	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER	PEC_{soil} (mg a.i. kg d.w.$^{-1}$)	TER
Triclopyr	CAR	*E. crypticus*	18 ± 21.3	0.02	356.7	ND	NC
	DAW	*E. crypticus*	76 ± 17.8	0.00	27950.0	ND	NC
	HJ1	*F. candida*	188 ± 74.3	0.16	662.5	0.04	4273
	HJ2	*F. candida*	161 ± 135	0.03	2434.4	ND	NC
Imazapyr	CAR	*E. crypticus*	392 ± 264	1.6	162	0.077	5091
	DAW	*E. crypticus*	23.3 ± 21.3	0.57	11.7	0.017	1371
	HJ1	*E. crypticus*	176 ± 116	0.001	71300	0.004	44000
	HJ2	*E. crypticus*	213 ± 108	0.002	44450	0.009	23667

NC: Not calculated
ND: Non-detect